National Library of Canada
Bibliothèque nationale du Canada

Canadian Theses Service
Service des thèses canadiennes

Ottawa, Canada
K1A 0N4

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, tests publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30.

NL-339 (r. 88/04)

Canada

THE UNIVERSITY OF ALBERTA

The Effect of Energy Intake, Body Fat Content and Food Deprivation on Hypothalamic Norepinephrine Metabolism

by

Barbara Marriage

©

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN

Nutrition

DEPARTMENT OF HOME ECONOMICS

EDMONTON, ALBERTA
FALL, 1987

Permission has been granted to the National Library of Canada to microfilm this thesis and to lend or sell copies of the film.

The author (copyright owner) has reserved other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without his/her written permission.

L'autorisation a été accordée à la Bibliothèque nationale du Canada de microfilmer cette thèse et de prêter ou de vendre des exemplaires du film.

L'auteur (titulaire du droit d'auteur) se réserve les autres droits de publication; ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation écrite.

ISBN 0-315-41182-1

THE UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR: Barbara Marriage

TITLE OF THESIS: The Effect of Energy Intake, Body Fat Content and Food Deprivation on Hypothalamic Norepinephrine Metabolism

DEGREE FOR WHICH THESIS WAS PRESENTED: Master of SCIENCE

YEAR THIS DEGREE GRANTED: FALL, 1987

Permission is hereby granted to THE UNIVERSITY OF ALBERTA LIBRARY to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly, or scientific purposes only.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

(SIGNED) ..... Barbara Marriage

PERMANENT ADDRESS:

7625-107 Street,
Edmonton, Alberta,
T6E 4X6

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled The Effect of Energy Intake, Body Fat Content and Food Deprivation on Hypothalamic Norepinephrine Metabolism submitted by Barbara Marriage in partial fulfillment of the requirements for the degree of Master of SCIENCE in Nutrition.

J Z Johnston
Supervisor

Ca. D. Clandinin

R T Coutts

Date July 21, 1987

Abstract

Hypothalamic norepinephrine (NE) synthesis rate during acute (1d and 3d) and chronic (11d) overfeeding (14 kcal/d) and underfeeding (7 kcal/d) and 24h food deprivation was determined to clarify the relationship between overfeeding, body fat content, food deprivation and brain noradrenergic activity. Four-week-old female mice (C57BL/6J, +/+) were underfed or overfed, in a meal-feeding paradigm, a 60% fat diet providing equal nutrient densities except for protein and carbohydrate. Constant protein intake was maintained across groups to minimize the variation in protein available for growth and to limit variability of tyrosine availability from dietary sources. NE synthesis rate was determined beginning at 20 minutes after presentation of a meal at the end of the dark period, during the thermic effect of the previous meal. NE synthesis rate was calculated from the rate of NE accumulation after monamine oxidase inhibition by pargyline and clorgyline. The ratio of tyrosine to neutral amino acids (tyrosine/NAA) in plasma was examined to determine if tyrosine availability influenced hypothalamic NE synthesis rate under these circumstances. Acute and chronic underfeeding versus overfeeding (chronic overfeeding resulting in a 36% difference in body fat content) had no effect on NE synthesis rate in the hypothalamus or in the rest of the brain. Similarly, chronic overfeeding did not affect NE synthesis rate in specific hypothalamic nuclei including suprachiasmatic, paraventricular (PVN), lateral, ventromedial (VMH), dorsomedial, anterior and arcuate nuclei. The VMN was the only hypothalamic area studied that showed a significant difference in NE concentration between underfed and overfed mice, with chronic overfeeding producing a 32.2% higher NE concentration. Although the acutely underfed mice had a lower tyrosine/NAA ratio, the NE synthesis rate did not differ

between the underfed and overfed mice. When mice were fed ad libitum or deprived of food for 24h, NE synthesis rate in the PVN and at no other site was five-fold higher in deprived mice. Thus, these results indicate that the thermic effects of overfeeding and underfeeding, and body fat content have no effect on hypothalamic NE synthsis rate in fed mice. The higher NE synthesis rate in the PVN of food-deprived mice is consistent with previous pharmacological evidence that an increase in noradrenergic activity in the PVN may be a stimulus for food ingestion.

Acknowledgements

I would like to thank my supervisor Dr. J.L. Johnston for her advice, support and constant enthusiasm throughout the research project; the members of my committee, Dr. M.T. Clandinin and Dr. R. Coutts, for their careful review of this thesis; A. Balachandran, for his expertise in laboratory methods and his patience in answering my endless questions; and David Hall for his careful typing and editing of this manuscript.

To my friends and colleagues, I would like to express my gratitude for their support, friendship, and sense of humour; a special thanks to Ron Stoyko and Linda McCargar for their endless support and encouragement.

TABLE OF CONTENTS

	Page
I. Introduction	1
A. Physiological significance of norepinephrine metabolism	2
B. Role of brain norepinephrine in food intake regulation	9
C. Role of food deprivation and feeding on brain norepinephrine synthesis	11
D. Role of brain norepinephrine in energy expenditure	13
E. Relationship of brain norepinephrine with body fat content	15
F. Summary	16
G. Objectives of the present study	17
H. References	19
II. The Effect of Energy Intake, Body Fat Content and Food Deprivation on Hypothalamic Norepinephrine Metabolism	23
A. Introduction	23
B. Methods	25
1. Animals and diets	25
2. Experimental protocol	27
3. Analytical methods	31
4. Statistical analyses	33
C. Results	33
D. Discussion and conclusions	52
E. References	61
III. Appendices	65
Appendix 1. Assay of norepinephrine	65
Appendix 2. Protein determination of hypothalamic nuclei	66
Appendix 3. Analysis of neutral amino acids by HPLC (o-phthaldialdehyde derivatives)	67

List of Tables

		Page
Table II-1	Diet composition	26
Table II-2	Effect of short-term (3d) underfeeding and overfeeding on change in weight and body fat content	34
Table II-3	Effect of short-term (3d) underfeeding and overfeeding on plasma tyrosine and tyrosine/NAA	35
Table II-4	Effect of short-term (3d) underfeeding and overfeeding on NE turnover in mouse brain	36
Table II-5	Effect of short-term (1d) underfeeding and overfeeding on plasma tyrosine and tyrosine/NAA	40
Table II-6	Effect of short-term (1d) underfeeding and overfeeding on NE turnover in mouse brain	40
Table II-7	Effect of chronic (11d) underfeeding and overfeeding on change in weight and body fat content (Exp.3)	43
Table II-8	Effect of chronic (11d) underfeeding and overfeeding on NE turnover in mouse brain	43
Table II-9	Effect of chronic (11d) underfeeding and overfeeding on change in weight and body fat content (Exp.4)	45
Table II-10	Coordinates used for the removal of specific hypothalamic nuclei from underfed, overfed, ad libitum and food-deprived mice and the protein content of each nucleus	46
Table II-11	Effect of chronic (11d) underfeeding and overfeeding on NE turnover in hypothalamic nuclei in mice	47
Table II-12	Effect of 24h food deprivation on NE turnover in hypothalamic nuclei in mice	50

List of Figures

		Page
Figure I-1	Hypothalamic regions	3
Figure I-2	Pathway of synthesis of norepinephrine	5
Figure I-3	Pathway of catabolism of norepinephrine	7
Figure II-1	Medial view of hypothalamic regions	28
Figure II-2	Accumulation of NE in mouse brain hypothalamus and rest of the brain after administration of pargyline and clorgyline in acutely (3d) underfed and overfed mice (Exp. 1)	36
Figure II-3	NE accumulation determination in mouse brain hypothalamus at 0, 10, 20, 30 and 40 min after administration of pargyline and clorgyline	37
Figure II-4	NE accumulation determination in mouse brain hypothalamus at 0, 20, and 40 min after administration of pargyline and clorgyline	38
Figure II-5	Accumulation of NE in mouse brain hypothalamus and rest of the brain after administration of pargyline and clorgyline in acutely (1d) underfed and overfed mice (Exp. 2)	41
Figure II-6	Accumulation of NE in mouse brain hypothalamus and rest of the brain after administration of pargyline and clorgyline in chronically (11d) underfed and overfed mice (Exp. 3)	44
Figure II-7	Accumulation of NE in specific hypothalamic nuclei after administration of pargyline and clorgyline in chronically (11d) underfed and overfed mice (Exp. 4)	48
Figure II-8	Accumulation of NE in specific hypothalamic nuclei after administration of pargyline and clorgyline in 24h food-deprived and fed mice (Exp. 5)	51

I. INTRODUCTION

Energy balance is normally a physiologically regulated process such that over time an individual's energy intake approximates expenditure and body fat content is defended. Energy balance is achieved when ingested food energy equals the energy expended plus that lost in feces, urine and as heat production. A clear examination of the mechanisms for weight gain and loss in animals reveals that variations in food intake are not always accounted for by differences in body fat or weight (1), suggesting the possibility of variable efficiency of energy utilization.

The ability of lean individuals to maintain energy balance on intakes that cause obesity in others suggests that the former possess a mechanism for disposal of unwanted food energy. Evidence for such a mechanism comes from a series of overfeeding experiments carried out on human volunteers which suggested that obesity could be a result of a thermogenic defect rather than a defect in food intake regulation (2).

A number of complex mechanisms have been proposed for the regulation of food intake (3), many of which have suggested a central role for the hypothalamus. The demonstration that intrahypothalamic injection of the catecholamine neurotransmitter, norepinephrine (NE), induces feeding, and that moderate depletion of brain NE causes a reduction in ad lib food intake in rats, suggests a role of brain NE in the facilitatory control of feeding (4).

When examining the role of output in the regulation of energy balance, it is usual to divide expenditure into several components. These components include obligatory and facultative thermogenesis. Obligatory thermogenesis is that due to the basal metabolic rate, the thermic affect of food, and voluntary activity (5). Facultative or regulatory thermogenesis is the energy expended in

excess of the obligatory demands and includes non-shivering thermogenesis (NST) and diet-induced thermogenesis (DIT). DIT is defined as the long-term adjustment in metabolic rate and heat dissipation following overfeeding (1). It is considered a facultative thermogenesis, because this mechanism operates to resist weight gain and obesity during overfeeding, and becomes inactive to help conserve body energy during periods of food restriction and starvation (5).

Catecholamines may play a critical role in the regulation of energy balance. The brain catecholamine norepinephrine (NE) has been implicated in many functions related to energy balance, as a stimulus for food intake (4) and for thermogenesis in brown adipose tissue (2).

The objective of the literature review is to provide a description of brain NE metabolism and an overview of the physiological significance of brain NE metabolism, particularly in the hypothalamus and hypothalamic nuclei. It will also focus on the role of brain NE in the regulation of energy balance, specifically by studying the relationships between energy intake, energy expenditure, body fat content and brain NE synthesis rate.

A. Physiological Significance of Norepinephrine Metabolism

The hypothalamus constitutes less than one percent of the total volume of the brain, yet it contains a large number of neuronal circuits concerned with vital functions. NE and a variety of signals are involved in the functioning of these circuits by controlling the regulation of temperature, heart rate, blood pressure, blood osmolarity, mood, and water and food intake (6). Different regions of the hypothalamus are specialized for different functions (Figure I-1).

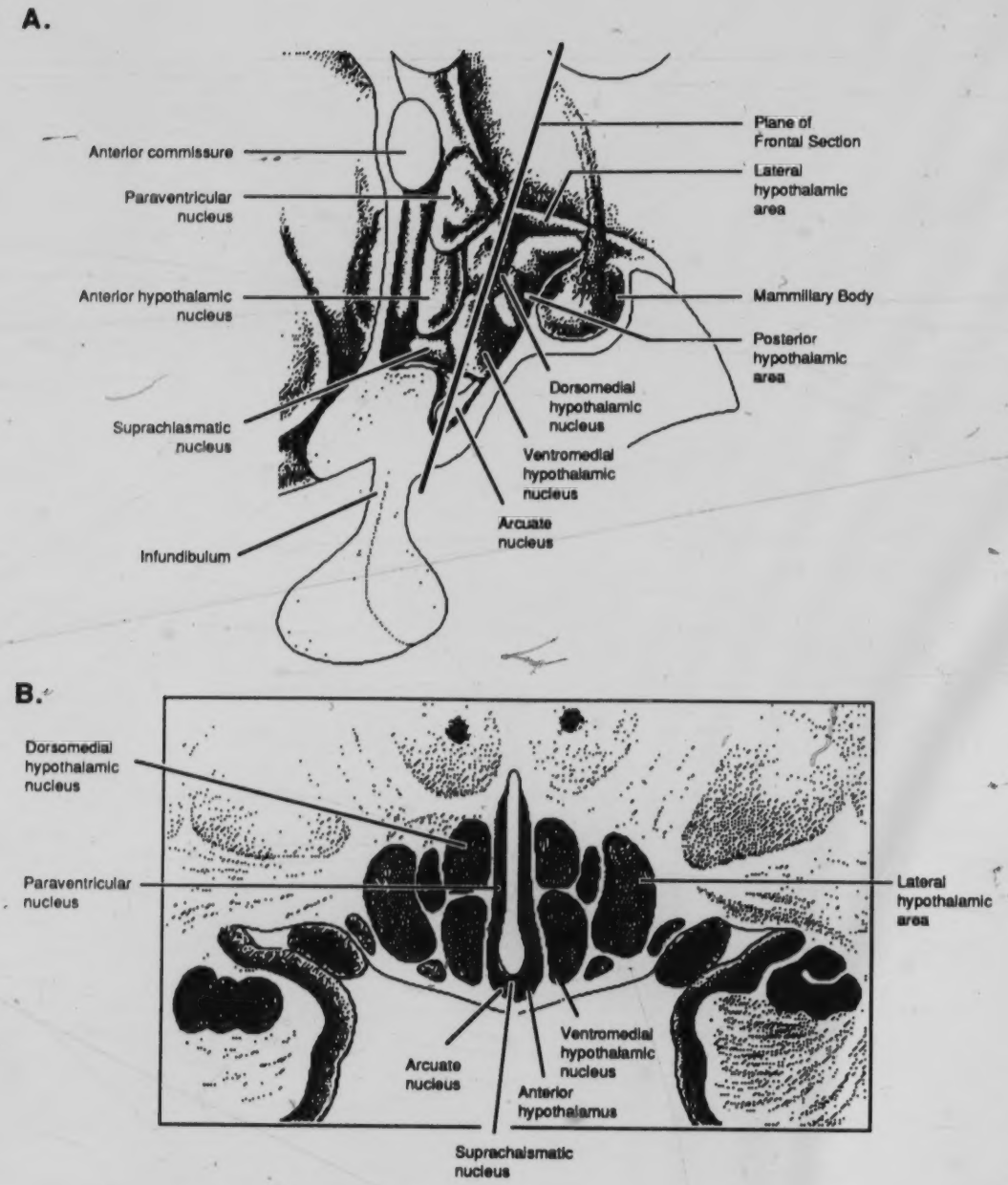

Figure I-1. Hypothalamic regions

A. Medial view of the hypothalamus

B. Frontal view (section along plane shown in A)

(adapted from Kupferman, I. (1985) Hypothalamic and limbic system I: peptidic neurons, homeostasis and emotional behavior. In: Principles of Neural Science (Kandel, E.R., Scwartz, J.H., eds.) Elsevier Science Publishing Co., New York)

The dorsal part of the hypothalamus, including the lateral hypothalamus (LH), paraventricular nucleus (PVN) and the dorsomedial nucleus (DM), has been ascribed a stimulatory function in feeding behavior, whereas the ventral part of the hypothalamus including the ventromedial nucleus (VMH) and the arcuate nucleus (ARC) has been attributed an inhibitory function, since lesions of the dorsal and ventral part result in, respectively, hypophagia and hyperphagia in rats (7). The anterior hypothalamus (ANT) is involved in body temperature regulation, with electrical stimulation of this area causing heat dissipation (6). The presence of a circadian mechanism in the suprachiasmatic nucleus (SCH) provides rhythmicity of corticosteriod release, feeding, drinking, locomotor activity and several other responses (6).

Neurotransmitters such as NE are the chemical links for neural communication among cells and appear to influence behavior by acting on the cells of their target tissues. NE neurotransmission may be influenced at one or more steps on the metabolic pathway, including synthesis, release, receptor binding, reuptake and catabolism. The first potentially influential step is the uptake of tyrosine, the amino acid precursor to NE, across the blood-brain barrier. Fluctuations in tyrosine concentrations may influence NE synthesis, because the rate-limiting enzyme in NE synthesis, tyrosine hydroxylase is unsaturated at physiological levels of its substrate (8). Once in the neuron, tyrosine is hydroxylated to dihydroxyphenylalanine (DOPA) by tyrosine hydroxylase. DOPA is rapidly decarboxylated to dopamine (DA) in a reaction catalyzed by aromatic L-amino acid decarboxylase. Dopamine-β-hydroxylase hydroxylates dopamine to form NE within storage vesicles (9) (Figure I-2). When a stimulus is sufficiently large enough to cause an action potential to travel down a neuron, NE is released from the nerve terminal into an extraneuronal gap, called a synapse. Once in the synapse, NE binds with

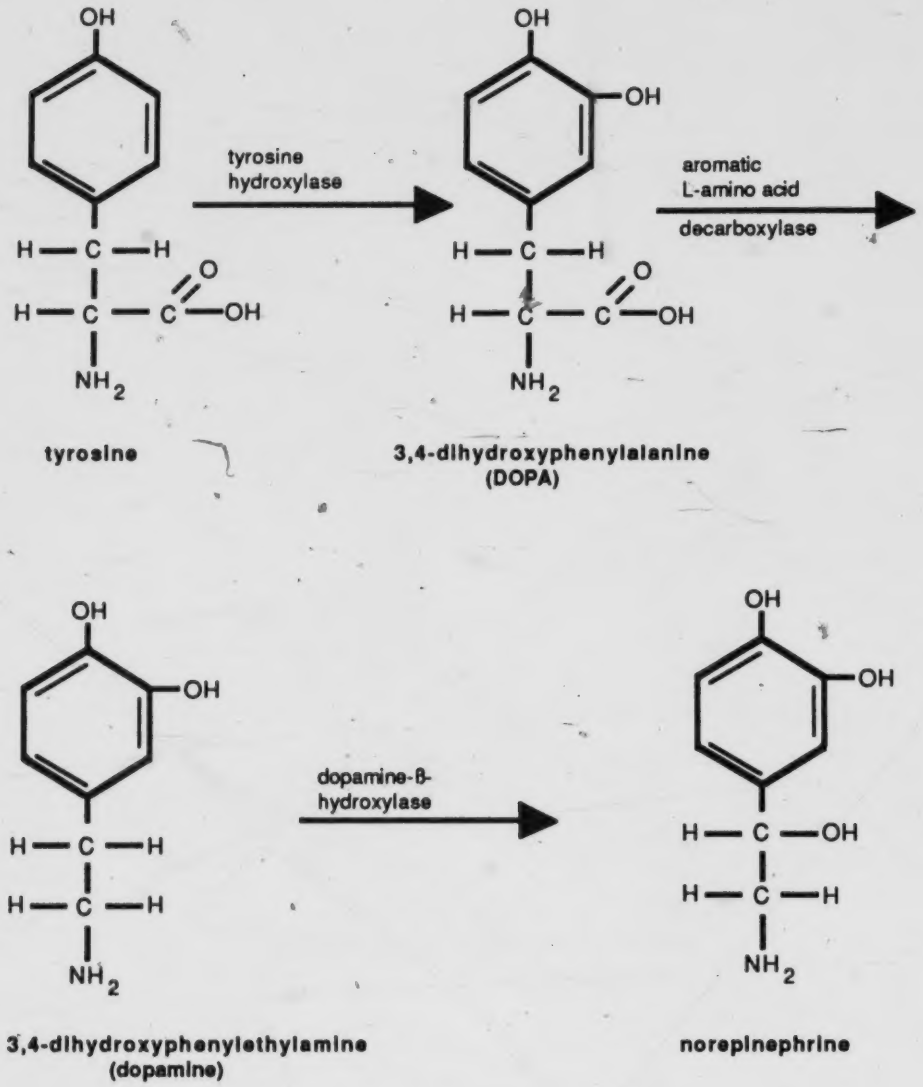

Figure I-2. Pathway of synthesis of norepinephrine

receptors on pre- and post-synaptic neurons to produce biological responses. The adrenergic receptors are divided into two main classes α and β, based on differences in their sensitivities to various agonists and antagonists (10). The physiological responses mediated by α-receptors in some tissues are different or opposite to those mediated by β-receptors, but in other tissues the responses are similar. β_1 and α_1 are normally postsynaptic in nature, whereas β_2 and α_2 are predominantly located presynaptically in the sympathetic terminal junction (11).

A major portion of the NE released undergoes reuptake, some of which is revesiculated and some is further catabolized. The catabolism of NE involves primarily two enzymes: monamine oxidase (MAO) and catechol-O-methyltransferase (COMT). In addition to these two major enzymes, an aldehyde oxidase and an aldehyde reductase act on the products of MAO and COMT. COMT is present mainly outside the sympathetic neuron, in contrast to MAO which has an important role in the regulation of the intracellular level of NE (12).

MAO deaminates NE to 3,4-dihydroxyphenylglycolaldehyde, which is either reduced to 3,4-dihydroxyphenylglycol (DHPG) or oxidized to dihydroxymandelic acid (DOMA). These intermediary metabolites are methylated by COMT to form 3-methoxy-4-hydroxyphenylglycol (MHPG), a major end product of NE catabolism, and vanilmandelic acid (VMA), respectively (12). Since DOMA is found only in trace amounts in the brain, VMA is not a major metabolite in the central noradrenergic neuronal system (13). Another pathway of NE catabolism is extraneuronal, where NE is methylated by COMT to form noremetanephrine (NMN) and then further deaminated to MHPG (12) (Figure I-3).

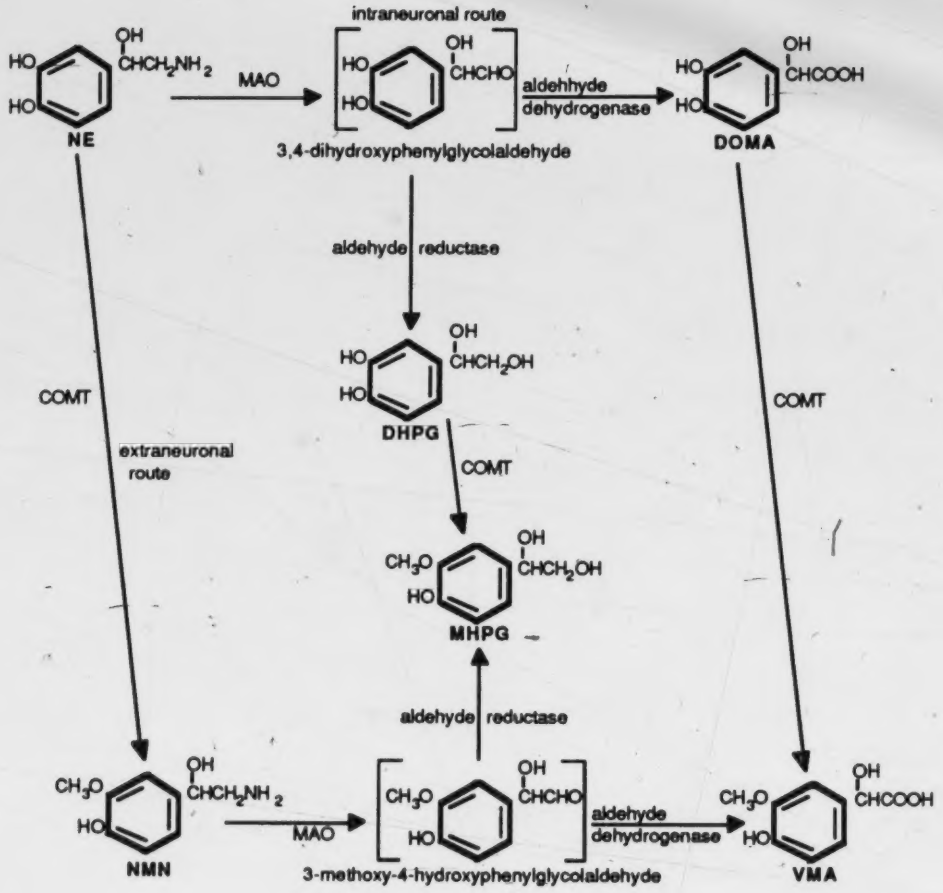

Figure 1-3. Pathway of catabolism of norepinephrine

NE - norepinephrine
DOMA - dihydroxymandelic acid
VMA - vanilmandelic acid
NMN - noremetanephrine
DHPG - 3,4-dihydroxyphenylglycol
MHPG - 3-methoxy-4-hydroxyphenylglycol
MAO - monamine oxidase
COMT - catechol-O-methyltransferase

Brain noradrenergic activity can be assessed by several methods. Measuring NE concentration in tissue represents the net content of NE, reflecting synthesis, release, reuptake and catabolism at one point in time, but does not reliably reflect the rate of flux through the pool (13). The measurement of NE metabolite levels, such as MHPG, may be a sensitive index of central NE turnover, but there are indications that these levels may be more sensitive to increases than to decreases in turnover (14). Hypothalamic NE is also measured by change in NE concentration after blockade of NE synthesis by α-methyl-para-tyrosine, at times ranging from 2 to 4 hours after α-methyl-para-tyrosine injection (15). The time course of the decline in NE concentration is necessary to investigate if the assumptions of steady state kinetics are met. Rate of accumulation of NE after inhibition of NE catabolism by the monamine oxidase (MAO) inhibitors pargyline and clorgyline is an alternative means of assessing NE turnover, and may be one of the most sensitive methods (16). Another method of assessment is to place cannulae in specific brain sites. Release of NE from cannula sites is quite sensitive to moment by moment changes in noradrenergic activity, but confirmation of the specific placement of cannulae is difficult (17). When analyzing or interpreting results of changes in NE concentration or NE turnover, the different techniques used to measure noradrenergic activity must be carefully examined. The failure of a stimulus to change NE concentration does not exclude the possibility of elevated or decreased turnover. Conversely, a change in NE concentration does not necessarily indicate change in turnover.

B. Role of Brain Norepinephrine in Food Intake Regulation

The classical theory of control of food intake is that there is a feeding center in the lateral hypothalamus and that if this center is damaged, aphagia occurs. In addition, there is a satiety center in the medial hypothalamus and if this is lesioned, hyperphagia occurs (6). More recently, the complexity of inputs to the hypothalamus has been recognized. The hypothalamus receives input from metabolic, hormonal, neurogenic, thermal and cortical factors describing the nutritional status of the organism. It has become evident that hypothalamic control of food intake involves a balance between a number of peptide neurotransmitters and the monamines (18). Centrally administered cholecystokinin (CCK) inhibits food intake in the rat and is considered a true satiety peptide (19). Glucagon, insulin, somatostatin, bombesin and calcitonin also decrease feeding after peripheral administration (20). Recent research has emphasized a role for the corticotropin-releasing factor (CRF) as a potent inhibitor of feeding. CRF exerts its effect on food intake when injected into the PVN, but not when injected into a number of other areas. This is the area within the brain where NE injections produce maximal stimulation of feeding (20). This suggests that NE increases food intake by suppressing the release of an inhibitory substance within the PVN.

Two classes of neuropeptides, namely the opioids and the pancreatic polypeptides (neuropeptide Y and peptide YY), acting via α_2-noradrenergic receptors have been associated with NE in the stimulation of feeding. Tests in different nuclei of the hypothalamus reveal strong sensitivity in the PVN to these endogenous substances (20). The neurogenic, hormonal, thermal and cortical factors may function both in association with and independently of

endogenous NE, depending upon brain area and the circumstances through which they become activated.

It is postulated that NE is involved in food intake regulation, as microinjections of NE into the PVN, and at no other site, acutely elicit feeding even in the satiated rat (21). Chronic NE injections into the PVN of rats induce a long-term increase in daily food intake and body weight gain (21). The demonstration that direct intrahypothalamic injections of NE can elicit feeding in rats strongly implicates NE in the neurochemical processes affecting feeding behaviour. The fact that feeding is reliably reinstated by intrahypothalamic NE administration, which would not be expected to stimulate peripheral NE receptors, suggests the converse possibility that the inhibition of feeding produced by pharmacological depletion of NE (4) is at least partially due to central NE depletion.

If facilitation of feeding is dependent on the release of NE, then manipulations which reduce brain NE activity should inhibit feeding. Data from such experiments have not consistently supported a role for brain NE as a facilitator of the feeding response. Blockage of α-adrenergic receptors inhibits feeding induced by central NE administration (22,23), but Broekkamp *et al.* (1974) demonstrated that α-adrenergic blockers inhibited consumption of food in the satiated rat, yet failed to do so in animals deprived of food for 24 hours (22). This evidence indicates that NE facilitates feeding but may not be the sole stimulus for food intake, particularly in the food-deprived animal. Food intake is a complex function of a number of external and internal signals together and the role of the noradrenergic system on naturally-occurring feeding behavior remains unclear.

C. Effect of Food Deprivation and Feeding on Brain Norepinephrine Synthesis

If increased brain noradrenergic activity facilitates feeding, and decreased noradrenergic activity inhibits feeding, the activity of the hypothalamic noradrenergic system and consequent NE turnover should tend to vary as a function of food deprivation and feeding, such that deprived animals have a high NE turnover and fed animals have a relatively low NE turnover. Such an association has been found between the activity of NE neurons in the hypothalamus and central glucoprivation (24). When glucoprivation is induced in rats by a subcutaneous insulin injection, hypothalamic NE turnover (as measured by decline in transmitter concentration after synthesis inhibition using α-methyl-para-tyrosine) is increased (24). Hypothalamic NE turnover in these rats returns to control levels after feeding. In the telencephalon, midbrain and brain stem regions, NE concentrations were not affected by insulin administration or by food intake, either during or after glucoprivation (24), indicating that the hypothalamus is the specific region for NE-induced feeding. Increased NE turnover in rats made hungry by insulin-induced glucoprivation is followed by the onset of feeding. Permitting the animals to eat a small amount of food either during or after the hypoglycemic period abolishes both increased NE turnover and further feeding (24).

The effect of chronic food deprivation on brain NE concentrations and turnover has yielded conflicting results (25,26,27). Glick *et al.* (1973) observed that food deprivation for 48 hours produced a lower hypothalamic NE concentration, relative to ad libitum fed controls, which may be indicative of increased NE turnover. Striatal dopamine (DA) concentration and NE concentration in the rest of the tel-diencephalon were not affected (25). Similarly, Stachowiak *et al.* (1976), using the punch technique for micro-

dissection of individual hypothalamic nuclei (26), found a decrease of NE and DA concentration in the ventromedial nucleus (VMH) in 48h food-deprived rats. There were no changes in the concentration of NE and DA in the lateral hypothalamic area (LH), dorsomedial nucleus (DM) or medial preoptic area (26). Loullis *et al.* (1979) found no changes in catecholamine concentration in any of the brain areas studied (which included the LH and VMH) in 48h food-deprived rats (27). However, significantly higher concentrations of the indoles serotonin and 5-hydroxyindole acetic acid, in the raphe nuclei and the LH, but not the VMH, were observed in the food-deprived rats (27). These changes in indole levels suggest that specific nuclei within the hypothalamus are differentially affected by food deprivation. These results do not necessarily mean that no changes in the metabolism of NE and DA take place following food deprivation, because tissue concentrations of catecholamines do not reliably reflect turnover. To further confuse the issue, food deprivation for 22h was reported to increase hypothalamic NE turnover (assessed by the decline in NE concentration four hours after α-methyl-para-tyrosine), while no changes occurred in hypothalamic NE concentration (29). Conversely, Pirke and Spyra (1982) reported no effect of 22h food deprivation on NE turnover (assessed at three hours after α-methyl-para-tyrosine injection) but a reduction in basal hypothalamic NE turnover after 48h food deprivation (30).

Catecholamine release before and during feeding has also been determined using a push-pull perfusion technique, a sensitive method for the continuous measurement of endogenous NE and DA release from specific areas within the hypothalamus in unanesthetized animals (31). In rats deprived of food for 16h, then subsequently allowed free access to food, release of NE from the VMH was consistently higher in the food-deprived rats measured at ten-minute intervals prior to food presentation than during satiety. During

feeding, NE release from the dorsomedial and perifornical areas was significantly higher than pre- and post-feeding values (31). The results of this study indicate that NE-containing neurons associated with the dorsomedial and perifornical areas of the hypothalamus are implicated in the maintenance of feeding and NE-containing neurons in the VMH are associated with hunger. In a subsequent study, Van Der Gugten *et al.* (1977) observed an increase in NE turnover in the rostral hypothalamic area and the dorsomedial areas in rats who exhibited a high feeding rate (32).

Clearly, the effects of food deprivation on hypothalamic noradrenergic activity are contradictory. A possible explanation for discrepancies is the different techniques used to measure noradrenergic activity. To complicate the issue further, whole hypothalami as well as specific nuclei have been examined and specific nuclei within the hypothalamus are differentially affected by food deprivation. Thus, the effect of food deprivation and feeding on brain NE turnover remains unclear.

D. Role of Brain Norepinephrine in Energy Expenditure

During overfeeding, some animals and some humans show an increase in energy expenditure that seems to compensate for the excess food intake and to minimize body weight gain. Noradrenergic activity in the ventromedial nucleus (VMH) of the hypothalamus is postulated to be involved in this energy expenditure via facultative thermogenesis. It has been suggested that the VMH is activated by an increase in noradrenergic activity in the fed state and this results in both satiety and diet-induced thermogenesis (DIT). Conversely, it has been proposed that the lateral hypothalamus (LH) is activated during fasting and would stimulate appetite but inhibit thermogenesis (33). In support

of the above hypothesis, it has been demonstrated that electrical stimulation of the VMH causes lipolysis in brown adipose tissue (BAT) of rats, as detected by a rise in plasma glycerol concentration, whereas electrical stimulation of the LH has no effect (34). This effect is mediated by sympathetic innervation of BAT. The VMH acts as a regulatory center for lipolysis in adipose tissue by modulating activation of the sympathetic nervous system (35). BAT has abundant direct innervation with noradrenergic fibers, whereas white adipose tissue has no direct sympathetic innervation; consequently its NE content is much lower than in BAT (34). Alterations in noradrenergic activity in the VMH may be a mediator of the effect of VMH stimulation of lipolysis in BAT. Furthermore, the intraventricular injection of NE increases plasma free fatty acids (FFA) and glycerol concentration (36). This response to intraventricular NE injection is blocked by propranolol, a β-antagonist which crosses the blood brain barrier, indicating the involvement of a β-adrenergic mechanism in the centrally mediated lipolysis activation (36).

If noradrenergic activity in specific brain areas such as the VMH is a stimulus for DIT, one would expect that overfeeding in a normal animal would result in a higher NE turnover in that brain region. Levin *et al.* (1986) found no such increase in the VMH of rats who were chronically overfed for three months, but an increased turnover of NE in the paraventricular nucleus (PVN) and dorsomedial nucleus (DM) (measured over three hours after α-methyl-para-tyrosine administration) (37).

The majority of recent research has focused on the peripheral sympathetic nervous system (SNS) as a mediator of thermogenesis after a meal and after chronic overfeeding. Chronic overfeeding of sucrose or a mixed palatable "cafeteria" diet increases NE turnover rate in heart and intrascapular

BAT in the rat (38), and modest overfeeding in normal human subjects has been shown to increase NE appearance rate in plasma, consistent with increased SNS activity (39).

If brain regional NE varies in response to short-term changes in energy intake in normal weight subjects, with subsequent activation or suppression of peripheral sympathetic activity, these changes may partially explain why some individuals maintain a relatively constant body weight despite large variations in food intake.

E. Relationship of Brain Norepinephrine Metabolism with Body Fat Content

Brain NE metabolism may also be related to body fat content. Genetically obese rodents show lower rates of NE turnover in brain (40) and BAT (41) than their lean counterparts, supporting the hypothesis that obesity may be related to a thermogenic defect. In humans, evidence for the relationships between brain NE metabolism and body fat content is indirect, but indicates that NE metabolite excretion is altered in obesity. Catecholamine metabolite excretion in women with normal body fat content has been compared with that of obese women (42). A urinary metabolite of NE, 3-methoxy-4-hydroxyphenylglycol (MHPG), is derived from both central and peripheral sources with the brain contributing between 20 and 60% (43). In normal weight women, the excretion of urinary MHPG is directly correlated with body fat content and is not related to energy intake. The obese women show an elevated MHPG excretion which is unrelated to energy intake or percent of body fat (42). The abnormality in catecholamine metabolism in the obese has been further supported by the work of Bazelmans *et al.* (1985). In their study of six obese males, the absence of any reduction of NE metabolism with under-

feeding, and an insignificant rise in NE appearance rate with overfeeding, suggests that peripheral sympathetic responsiveness to changing energy states is blunted in the obese (44).

NE metabolism is also altered when body fat content is low. In women with anorexia nervosa, the excretion of NE metabolites including MHPG is low, and refeeding for one week does not result in normal quantities of urinary catecholamine metabolites (45). Furthermore, the reduced catecholamine metabolite excretion in underweight women only returns to normal when refeeding has resulted in weight gain to normal levels (45).

These results suggest that both central and peripheral NE metabolism may be altered when body fat content is abnormally high (as in obesity) or extremely low (as in anorexia nervosa), an effect that appears to be independent of energy intake. It is difficult to address the question of central NE involvement in humans, but results suggest a direct relationship between brain NE metabolism and body fat content in normal weight and underweight women independent of energy intake.

F. Summary

In conclusion, brain noradrenergic activity appears to be involved in several components of energy balance, including both food intake and energy expenditure. Although the evidence is consistent that NE enhances food intake acutely and weight gain chronically, the effects of NE depletion on food intake and weight are inconsistent. Furthermore, glucoprivation induced by insulin increases hypothalamic NE turnover, while reports of the effects of food deprivation on brain regional NE turnover are inconsistent. NE in specific hypothalamic regions may be involved in the initiation of food intake, with

synthesis rate increased prior to a meal and reduced after a meal. This pattern of noradrenergic activity is not compatible with the hypothesis that an increase in brain noradrenergic activity is a stimulus for thermogenesis. If NE is a key regulator of energy balance, one would expect an increased NE synthesis rate after overfeeding and reduced NE synthesis rate after food deprivation or underfeeding. It is possible that two or more hypothalamic sites are involved in food intake and thermogenesis and respond differentially to feeding. To examine this possiblility, a study of NE turnover in specific hypothalamic nuclei is warranted.

Alteration in brain NE metabolism is seen in genetically obese animals, and urinary excretion of NE metabolites is decreased in obese women. Whether these abnormalities in NE metabolism are a cause or a result of obesity has not been determined. It is important to clarify the relationships between food deprivation, overfeeding, body fat content and brain noradrenergic activity, because the implications are substantial for the treatment of human obesity.

G. Objectives of the Present Study

The objectives of this study were to investigate the relationships between energy intake, food deprivation, body fat content and brain noradrenergic activity in normal mice. It is hypothesized that defense of body fat content is of primary importance in the regulation of energy balance. Therefore, it is hypothesized that acute underfeeding will decrease, and acute overfeeding will increase NE turnover, and this will be apparent at the level of the whole hypothalamus. Second, when chronic overfeeding in an otherwise normal animal results in a stable elevated body fat content, it is hypothesized

that NE turnover in the overfed animal will be higher than in the underfed animal, and this will be apparent at the level of the whole hypothalamus and in the ventromedial nucleus (VMH) specifically. Third, it is hypothesized that NE in the paraventricular nucleus (PVN) may be involved in the initiation of food intake with NE synthesis rate higher in food-deprived animals than in fed animals.

H. References

1. Rothwell, N.J., and Stock, M.J. (1981) Regulation of energy balance. Ann. Rev. Nutr. *1*, 235-257.

2. Rothwell, N.J., and Stock, M.J. (1981) Diet-induced thermogenesis: a role for brown adipose tissue. In: Nutritional Factors: Modulating Effects on Metabolic Processes, (Beers, R.F., Bassett, E.G., eds) pp. 101-113, Raven Press, New York.

3. Van Itallie, T.B., Gale, S.K., and Kissileff, H.R. (1978). Control of food intake in the regulation of depot fat: an overview. In: Advances in Modern Nutrition (Katzen, H.M., Mahler, R.J., eds.) pp. 427-492, Hemisphere Press, London.

4. Rossi III, J., Zolovick, A.J., Davis, R.F., and Pansksepp, J. (1982) The role of norepinephrine in feeding behavior. Neurosci. Biobehav. Rev. *6*, 195-204.

5. Himms-Hagen, J. (1981) Non-shivering thermogenesis, brown adipose activity and obesity. In: Nutritional Factors: Modulating Effects on Metabolic Processes, (Beers, R.F., Bassett, E.G., eds) pp. 85-99, Raven Press, New York.

6. Kupferman, I. (1985) Hypothalamus and limbic system I: peptidergic neurons, homeostasis and emotional behavior. In: Principles of Neural Science (Kandel, E.R., Schwartz, J.H., eds.) pp. 612-635, Elsevier Science Publishing Co., New York.

7. Leibowitz, S.F., Hammer, N.J. and Brown L.L. (1980) Analysis of behavioral deficits produced by lesions in the dorsal and ventral midbrain tegmentum. Physiol. Behav. *25*, 829-843.

8. Anderson, G.H. and Johnston, J.L. (1983) Nutrient control of brain neurotransmitter synthesis and function. Can. J. Physiol. Pharmacol. *61*, 271-281.

9. Sved, A.F. (1983) Precursor control of the function of monoaminergic neurons. In: Nutrition and the Brain, Vol. 3 (Wurtman, R.J., Wurtman, J.J., eds.) pp. 223-275, Raven Press, New York.

10. Exton, J.H. (1981) Mechanisms involved in α-adrenergic effects of catecholamines. In: Adrenoceptors and Catecholamine Action, Vol. 1 (Kunos, G., ed.) pp. 117-129, John Wiley and Sons, Inc., New York.

11. Areins, E.J. and Simonis, A.M. (1983) Physiological and pharmacological aspects of adrenergic receptor classification. Biochem. Pharmacol. *32*, 1539-1545.

12. Molinoff, P.B. and Axelrod, J. (1971) Biochemistry of catecholamines. In: Annual Review of Biocheminstry, Vol. 40 (Snell, E.E., Boyer, P.D., Meister, A., Sinsheimer, R.L., eds.) pp. 465-493, Annual Reviews Inc., Palo Alto, California.

13. Esler, M. (1982) Assessment of sympathetic nervous function in humans from noradrenaline plasma kinetics. Clin Sci *62*, 247-254.

14. DeMet, E.M. and Halaris, A.E. (1979) Origin and distribution of 3-methoxy-4-hydroxyphenylglycol in body fluids. Biochem. Pharmacol. *28*, 3043-3050.

15. Brodie, B.B., Costa, E., Dlabec, A., Neff, N.N. and Snookler, H.H. (1966) Application of steady-state kinetics to the estimation of synthesis rate and turnover time of tissue catecholamines. J. Pharmocol. Exp. Ther. *154*, 493-498.

16. Li, P.P., Warsh, J.J. and Godse, D.D. (1984) Formation and clearance of norepinephrine glycol metabolites in mouse brain. J. Neurochem. *43*, 1425-1433.

17. Myers, R.D. (1972) Methods for perfusing different structures of the brain. In: Methods in Psychobiology, Vol. 2 (Myers, R.D. ed.) pp. 169-211, Academic Press, New York.

18. Morley, J.E. (1980) The neuroendocrine control of appetite: the role of the endogenous opiates, cholecystokinin, TRH, gamma-amino-butyric-acid and diazepem receptor. Life Sci. *27*, 355-368.

19. Crawley, J.N. and Knas, J.Z. (1984) Tracing the sensory pathway from gut to brain regions mediating the actions of cholecystokinin of feeding and exploration. Proc. Soc. Neurosci. Abstr. *10*, 533-534.

20. Morley, J.E., Gosnell, B.A., Krahn, D.D., Mitchell, J.E. and Levine, A.S. (1985) Clinical implications of neurochemical mechanisms of appetite regulation. Psychopharm. Bull. *21*, 400-405.

21. Liebowitz, S.F. (1978) Paraventricular nucleus: a primary site mediating adrenergic stimulation of feeding and drinking. Pharmacol. Biochem. Behav. *8*, 163-175.

22. Broekkamp, C.L.E., Honig, W.M.M., Pauli, A.I. and Van Rossum, J.M. (1974) Pharmocological suppression of eating behavior in relation to diencephalic noradrenergic receptors. Life Sci. *14*, 473-481.

23. Liebowitz, S.F. (1975) Ingestion in the satiated rat: Role of alpha and beta receptors in mediating effects of hypothalamic adrenergic stimulation. Physiol. Behav. *14*, 743-754,.

24. Bellin, S.I., and Ritter, S. (1981) Insulin-induced elevation of hypothalamic norepinephrine turnover persists after glucorestoration unless feeding occurs. Brain Res. *217*, 327-337.

25. Glick, S.D., Waters, D.H. and Milloy, S. (1973) Depletion of hypothalamic norepinephrine by food deprivation and interaction with D-amphetamine. Res. Commun. Chem. Pathol. Pharmacol. *6*, 775-778.

26. Stachowiak, M., Bialowas, J. and Jurkowski, M. (1978) Catecholamines in some hypothalamic and telencephalic nuclei of food-deprived rats. Acta Neurobiol. Exp. *38*, 157-165.

27. Loullis, C.C., Felten, D.L. and Shea, P.A. (1979) HPLC determinations of biogenic amines in discrete brain areas in food-deprived rats. Pharmcol. Biochem. Behav. *11*, 89-93.

28. Palkovits, M. (1973) Isolated removal of hypothalamic or other brain nuclei of the rat. Brain Res. *59*, 449-450.

29. Friedman, E., Starr, N. and Gershon, S. (1973) Catecholamine synthesis and the regulation of food intake in the rat. Life Sci. *12*, 317-326.

30. Pirke, K.M. and Spyra, B. (1980) Catecholamine turnover in the brain and the regulation of lutenizing hormone and corticosterone in starved male rats. Acta Endrocrinol. *100*, 168-172.

31. Van Der Gugten, J. and Slangen, JL. (1977) Release of endogenous catecholamines from rat hypothalamus in vivo related to feeding and other behaviors. Pharmocol. Biochem. Behav. *7*, 211-219.

32. Van Der Gugten, J., De Kloet, E.R., Versteeg, D.H.G. and Slangen, J.L. (1977) Regional hypothalamic catecholamine metabolism and food intake regulation in the rat. Brain Res. *135*, 325-336.

33. Rothwell, N.J. and Stock, M.J. (1982) Neural regulation of thermogenesis. Trends in Neurosci. *5*, 124-127.

34. Shimazu, T. and Takahashi, A. (1980) Stimulation of hypothalamic nuclei has differential effects on lipid synthesis in brown and white adipose. Nature *284*, 62-63.

35. Shimazu, T. (1981) Central nervous system regulation of liver and adipose tissue metabolism. Diabetologia *20*, 343-356.

36. Barbosa, M.C. and Migliorini, R.H. (1982) Free fatty acid mobilization in rats following intracerebroventricular norepinephrine. Am. J. Physiol. *242*, E248-E252.

37. Levin, B.E., Triscari, J. and Sullivan, A. (1985) The effect of diet and chronic obesity on brain catecholamine turnover in the rat. Pharmacol. Biochem. Behav. *24*, 299-304.

38. Young, J.B., Saville, E., Rothwell, N.J., Stock, M.J.and Landsberg, L. (1982) Effect of diet and cold exposure on norepinephrine turnover in brown adipose tissue of the rat. J. Clin. Invest. *69*, 1061-1071.

39. O'Dea, K., Esler, M., Leonard, P., Stockigt, J.R. and Nestel, P. (1982) Noradrenaline turnover during under- and over-eating in normal weight subjects. Metabol. *31*, 896-899.

40. Johnston, J.L., Romsos, D.R. and Bergen, W.G. (1986) Reduced brain norepinephrine metabolism in obese (*ob/ob*) mice is not normalized by tyrosine supplementation. J. Nutr. *116*, 435-445.

41. Knehans, A.W. and Romsos, D. (1982) Reduced norepinephrine turnover in brown adipose tissue of *ob/ob* mice. Am. J. Physiol. *242*, E253-E261.

42. Johnston, J.L., Warsh, J.J. and Anderson, G.H. (1983) Obesity and precursor availability affect urinary catecholamine metabolite production in women. Am. J. Clin. Nutr. *38*, 356-368.

43. Maas, J.W., Hattox, S.E., Green, N.M., and Landis, D.H. (1979) 3-methoxy-4-hydroxyphenylethylene glycol production by human brain in vivo. Science *205*, 1025-1027.

44. Bazelmans, J., Nestel, P.J., O'Dea, K. and Esler, M.D. (1984) Blunted norepinephrine responsiveness to changing energy states in obese subjects. Metabol. *34*, 154-160.

45. Johnston, J.L., Leiter, L.A., Burrow, G.N., Garfinkel, P.E. and Anderson, G.H. (1984) Excretion of urinary catecholamine metabolites in anorexia nervosa: effect of body composition and energy intake. Am. J. Clin. Nutr. *40*, 1001-1006.

II. EFFECT OF ENERGY INTAKE, BODY FAT CONTENT AND FOOD DEPRIVATION ON HYPOTHALAMIC NOREPINEPHRINE METABOLISM

A. Introduction

A number of complex mechanisms have been proposed for the regulation of food intake and energy expenditure, several of which have implicated the activity of catecholamine-containing neurons, specifically in the hypothalamus (1). The demonstration that intracerebroventricular injection of norepinephrine (NE) induces feeding, and that moderate depletion of brain NE causes a reduction in ad libitum food intake in rat, suggests that brain NE has a role in the facilitory control of feeding (2). Different regions of the hypothalamus are specialized for different functions, and the paraventricular nucleus (PVN) is the area within the brain where NE injections produce maximal stimulation of feeding (3).

If increased brain noradrenergic activity facilitates feeding, and decreased noradrenergic activity inhibits feeding, the activity of the hypothalamic noradrenergic system and consequent NE turnover should tend to vary as a function of food deprivation and feeding such that food-deprived animals have a relatively high NE turnover. Studies of the effect of food deprivation on noradrenergic activity have yielded conflicting results (4,5,6). NE concentration has been reported to be decreased (4,5) or unchanged (6) in the hypothalamus or in hypothalamic areas of 48h deprived rats. When turnover is assessed, 22h food deprivation has been reported to increase (7), or have no effect (8) on NE synthesis rate.

It is possible that the involvement of norepinephrine in the regulation of food intake and energy expenditure is site specific, and that in examining the whole hypothalamus, site specific responses are masked.

For example, the involvement of hypothalamic noradrenergic activity in diet-induced thermogenesis (DIT) is suggested by the evidence that electrical stimulation of the ventromedial nucleus (VMH) (9) and intraventricular injections of NE (10) cause lipolysis in the brown adipose tissue (BAT) of rats, as detected by a rise in plasma glycerol concentration and plasma free fatty acids (9,10). However, when noradrenergic activity in specific hypothalamic areas such as the VMH is examined as a possible site for the stimulation of DIT, no increase in NE turnover in the VMH of rats that were chronically overfed for three months is seen, but the turnover of NE in the PVN and dorsomedial nucleus (DM) is higher than in ad libitum fed counterparts (11). Thus, a clear demonstration of the role of NE in the hypothalamus or in hypothalamic nuclei in physiologically relevant conditions of hunger, and acute and chronic overfeeding is lacking.

Thus, the objectives of this study were to investigate the relationships between energy intake, body fat content, food deprivation and NE synthesis rate in the hypothalamus or hypothalamic nuclei of mice. First, the effects of short-term underfeeding and overfeeding on hypothalamic NE synthesis rate in meal-fed mice were examined. Second, the effects of chronic underfeeding and overfeeding (resulting in stable differences in body fat content) in meal-fed mice on NE synthesis rate in the hypothalamus and hypothalamic nuclei were investigated. Third, the effect of food deprivation on NE synthesis rate in specific hypothalamic nuclei was investigated. Last, the possible role of tyrosine availability in mediating observed differences was also investigated.

B. Methods

1. Animals and Diets

Four-week-old female mice (C57BL/6J, +/+, Jackson Laboratories, Bar Harbour, ME) were used in all experiments. A 12-hour light:dark cycle with lights on starting at 0900h was maintained throughout the experiments. The mice were housed individually in hanging wire-mesh cages in a temperature-controlled room (25±2°C). They had free access to water throughout the experiments. Mice were weighed daily between 0900 and 1000h. In all experiments, mice were adapted for one week to a purified diet, containing 20% of metabolizable energy as protein, 60% as carbohydrate and 20% as fat (Table II-1-control). In experiments 1 and 2, the mice were adapted to meal feeding the purified diet (Table II-1-control) in an amount equivalent to ad libitum intake (3 g/d), divided into six equal meals delivered by a small animal programmed feeder (Hofer, Switzerland). In experiments 1, 2, 3, and 4, after one week of adaptation, all mice were either underfed or overfed a high fat diet with equal quantities of protein to minimize the variation in protein available for growth. Another reason for maintaining constant protein intake across groups was to limit variability of tyrosine availability from dietary sources (12,13). The diets, except for protein and carbohydrate, had equal nutrient densities (Table II-1). Mice in the overfed group (Table II-1-high fat overfed) received an energy level of 59.6 kJ (14.2 kcal)/d with 0.7 g protein. This amount is equivalent to the ad libitum intake of protein and energy on this high fat diet (Johnston, J.L., unpublished observation) and equals 2.9 g/d. The high fat diet was chosen to promote vigorous overfeeding (14). Mice in the underfed group (Table II-1- high fat underfed) received an energy level of 30.7 kJ (7.3 kcal)/d with 0.7 g protein. This is equivalent to 51% of the energy intake of the overfed group and equals 1.5 g/d.

Table II-1.
Diet Composition (g/100 g)

Ingredients	Control	High fat control	High fat overfed	High fat underfed
Casein	23.0	34.3	27.7	52.8
Cellulose	4.0	5.5	5.5	5.4
Corn oil	5.0	16.4	16.4	16.2
Lard	5.0	16.4	16.4	16.2
Glucose	29.2	10.6	13.7	1.5
Starch	19.2	10.6	13.7	1.5
Mineral mix[1]	3.5	4.8	4.9	4.8
Vitamin mix[2]	1.0	1.4	1.4	1.4
Choline bitartrate	0.2	0.3	0.3	0.3
Protein (%)[3]	20.0	24.3	20.0	37.8
Carbohydrate (%)	60.0	15.7	20.0	2.2
Fat (%)	20.0	60.0	60.0	60.0
Energy Density[4]	15.5 kJ/g (3.7kcal/g)	20.6 kJ/g (4.9 kcal/g)	20.6 kJ/g (4.9 kcal/g)	20.6 kJ/g (4.9 kcal/g)

•1 AIN-76 mineral mixture (ICN Nutritional Biochemicals, Cleveland, OH). Composition in g/kg: calcium phosphate, dibasic 500; sodium chloride 74; potassium citrate, monohydrate 220; potassium sulfate 52; magnesium oxide 24; manganous carbonate 3.5; ferric citrate 6; zinc carbonate 1.6; cupric carbonate 0.3; potassium iodate 0.01; sodium selenite 0.01; chromium potassium sulfate 0.55; and sucrose 118.03.

•2 AIN-76 vitamin mixture (ICN Nutritional Biochemicals, Cleveland, OH). Composition in mg/kg: thiamin HCl 600; riboflavin 600; pyridoxine HCl 700; nicotinic acid 3000; D-calcium pantothenate 1600; folic acid 200; D-biotin 20; cyanocobalamin 1; retinyl palmitate 800; dl-a-tocopheryl acetate 20000; cholecalciferol 2.5; menaquinone 5; and sucrose 972474.5.

3 % of metabolizable energy

4 Based on 16.8(4), 16.8(4), and 37.8 kJ (9 kcal)/g metabolizable energy for protein from casein, carbohydrate from glucose and starch, and fat from corn oil and lard, respectively.

• Recommendations from *Report of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies.* (1976) J. Nutr. *107*, 1340-1348.

2. Experiment Protocol

Experiment 1. Mice in the underfed and overfed groups received their daily intake in six equal meals within each treatment, delivered by a programmed feeder starting at 2300h. A treatment duration of three days was chosen to allow familiarization to the high fat diet. Mice were killed beginning at 0920h, 20 min after presentation of the last meal for determination of NE synthesis rate. NE synthesis rate in the hypothalamus and the rest of the brain was assumed to reflect noradrenergic activity in satiated mice experiencing the thermic effect of the previous meal. NE synthesis rate was determined from the rate of accumulation of NE after inhibition of NE catabolism by the monamine oxidase inhibitors pargyline hydrochloride (Sigma Chemical Co., St. Louis, MO) (75 mg/kg) and clorgyline hydrochloride (generous gift of May and Baker Ltd., Dagenham, England) (10 mg/kg). Mice were killed by decapitation at 0, 20, and 40 min. after a single intraperitoneal (i.p.) injection of these inhibitors mixed in physiological saline (10 ml/kg). These MAO inhibitors were chosen because two catalytically distinguishable MAO activities have been found to be present in the mouse brain (15). MAO-A activity is sensitive to inhibition by low concentrations of clorgyline, whereas MAO-B is relatively sensitive to pargyline (15). The hypothalamus was dissected along natural lines of demarkation by taking the anterior commissure as the horizontal reference and the line between the posterior hypothalamus and the mammillary bodies as the caudal limit (16) (Figure II-1). The hypothalamus and the rest of the brain were immediately removed, weighed, wrapped in aluminum foil, frozen on dry ice and held at -40°C for subsequent determination of NE within one month. Blood was drawn from the cervical wound and centrifuged, and plasma was stored at -40°C until determination of neutral amino acids (NAA) within two months. The carcasses (minus the brain) were frozen until body fat content could be determined.

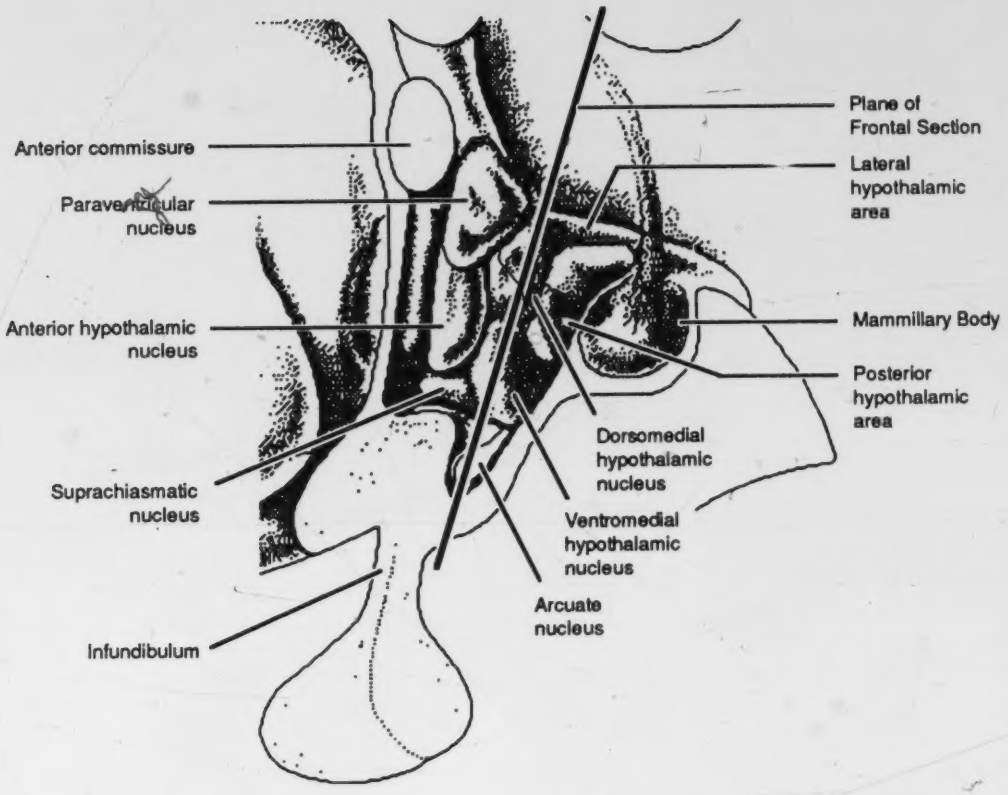

Figure II-1. Medial view of hypothalamic regions

(adapted from Kupferman, I. (1985) Hypothalamic and limbic system I: peptidic neurons, homeostasis and emotional behavior. In: Principles of Neural Science (Kandel, E.R., Scwartz, J.H., eds.) Elsevier Science Publishing Co., New York)

Experiment 2. Mice were underfed or overfed the high fat diets for one day to eliminate the confounding effect of weight changes, resulting from under- and over-feeding for three days. The six meals per day paradigm was repeated as in Exp. 1. At the end of the one-day experiment, mice were killed as in Exp. 1. The hypothalamus and the rest of the brain were dissected for subsequent determination of NE, and blood was collected for NAA analysis as in Exp. 1.

Experiment 3. Mice were underfed or overfed the high fat diet for eleven days to produce chronic changes in body weight and body fat content. Mice received the underfeeding and overfeeding diets in one meal per day presented at 1600h until the last day of the experiment when the mice received their daily intake minus one meal (equivalent to one-sixth of the daily intake). The final meal was presented at 0900h on the day of the killing. Mice were killed beginning at 0920h for determination of NE synthesis rate as in Exp. 1. The hypothalamus and the rest of the brain were dissected for subsequent determination of NE as in Exp. 1 and 2. The carcasses (minus the brain) were frozen until body fat content could be determined.

Experiment 4. Mice were underfed and overfed the high fat diets for eleven days following the same feeding paradigm as in Exp. 3. Mice were killed by decapitation at 0 and 40 min. after a single i.p. injection of pargyline hydrochloride and clorgyline hydrochloride, mixed in physiological saline as described in Exp. 1. Whole brains were removed, wrapped in aluminum foil, frozen on dry ice and held at -70°C for processing within one month. For the microdissection of hypothalamic nuclei, the frozen brain was mounted on a specimen holder using an embedding medium (Tissue-Tek, O.C.T. Compound,

Miles Scientific, Naperville, IL) and then placed in a histostat (Model 855, Reichert-Jung Scientific Instruments, Belleville, Ont.) maintained at -5 to -8°C. Frozen sections measuring 300 μm were prepared, using the orientations of the stereotaxic atlas of Slotnick and Leonard (17). The frozen sections were mounted on glass microscope slides by partially thawing the section on the slide, followed by immediate refreezing on dry ice. The slides were placed on a cold plate (Stir-Kool, Model SK12, Thermo Electric Co. Inc., Saddle Brook, NJ) and the suprachiasmatic nucleus (SCH), anterior hypthalamus (ANT), paraventricular nucleus (PVN), ventromedial nucleus (VMH), dorsomedial nucleus (DMN), lateral hypothalamus (LH), and the arcuate nucleus (ARC) were removed using a stainless steel needle with an inside diameter of 430 μm (NIH Style Neuropunch, Fine Science Tools, Vancouver, B.C.). The atlas of Slotnick and Leonard (17) was used as a guide to locate the nuclei. The removal of the appropriate nuclei was confirmed after microdissection by visual inspection of each slide after partial thawing. Upon removal of the nuclei, the tissue was immediately placed in 60μl of 0.1 N perchloric acid ($HClO_4$) and stored at -70°C for subsequent NE analysis. The carcasses (minus the brain) were frozen until body fat content could be determined.

Experiment 5. The mice were fed for seven days ad libitum a high fat diet (Table II-1-high fat control). Half of the mice continued to be fed ad libitum and half were deprived for 24 h before killing. Mice were killed beginning at 0900h at 0 and at 40 min after a single i.p. injection of pargyline and clorgyline. Brains were removed, and hypothalamic nuclei were microdissected as in Exp. 4 and frozen at -70°C for subsequent NE determination.

NE accumulation determination. In all experiments, NE accumulation was measured over at least 0 to 40 min. Two subsequent experiments were conducted to determine the time course for the best linear fit of NE accumulation following catabolism inhibition.

NE accumulation was measured at 0, 10, 20, 30 and 40 min in the hypothalamus after one week of adaptation to the high fat diet (Table II-1-high fat control). NE accumulation appeared to peak at 20 min, but not definitively so. The experiment was repeated with mice killed at 0, 20 and 40 min, and the data of the two experiments were pooled at 0, 20 and 40 min. The resulting data best fit a polynomial equation, i.e. the accumulation was not linear beyond 20 min. Therefore, statistical comparisons between slopes of lines were made where accumulation was most linear, i.e. over 0 to 20 min in the short-term feeding experiments (Exp. 1 and 2). In the chronic feeding experiments, the accumulation was linear for at least 40 min. Thus, in Exp. 3 and 4, and for comparison of the nuclei in Exp 5, the rate of NE synthesis and comparison of the slopes of the lines were determined from the increase over 40 min, measured at 2 time points.

3. Analytical Methods

NE in the whole hypothalamus was isolated by alumina extraction from the tissue homogenate supernatant under alkaline conditions by a previously reported method (18) (Appendix 1). For NE determination in the individual hypothalamic nuclei, frozen tissue in 60 µl 0.1 M perchloric acid ($HClO_4$) was thawed and 10µl 0.2 M ethylenediamine tetraacetic acid (EDTA), 5 µl 1.0 M sodium bisulfite ($NaHSO_4$) and 30 µl .0007 M 3,4-dihydroxybenzylamine (DHBA), an internal standard, were added. The samples were sonicated (Model 300, Sonic Dismembrator, Artek Systems Corp., Pharmingdale, NY) for

20 sec, and microcentrifuged at 9000 rpm for two min, and the supernatant was injected onto a liquid chromatographic system for detection.

Detection of NE was achieved by high performance liquid chromatography (HPLC) (Model 2000, Varian Canada Inc., Georgetown, Ont.) with electrochemical detection (EC) (Model LC4, Bioanalytical Systems Inc., West Lafayette, IN) using a mobile phase of 0.075 M monochloroacetic acid with 250 mg/l sodium octyl sulfate as an ion-pairing reagent. The mobile phase was delivered at room temperature at a flow rate of 1.7 ml/min. across a reversed-phase analytical column. The limit of detection in hypothalamic nuclei was 0.10 ng of NE. The between-run coefficient of variation on freshly prepared processed standard relative to internal standard was 3.6%. The protein content of the hypothalamic nuclei was determined by a modified Lowry procedure (19) on the resuspended pellet of the centrifuged homogenate (Appendix 2). Values are expressed as pg of amine per µg of protein.

Separation and quantification of the individual neutral amino acids, valine, leucine, isoleucine, tryptophan, phenylalanine and tyrosine were accomplished by HPLC with fluorometric detection by a previously reported method (20) (Appendix 3).

Body fat composition was determined after removing food residue from the stomach. The carcass (minus the brain) was homogenized (Polytron™, Brinkman Instruments, Westbury, NY) in an equal weight of water. Body fat was determined from dried aliquots of homogenized carcasses by ether extraction on a Goldfinch apparatus and quantitated gravimetrically (21).

4. Statistical Analyses

NE synthesis rate was calculated by linear regression of NE concentration versus the time periods specified, and the slopes of the regression lines were compared using the variance estimated for the difference between slopes (22). Treatment differences for NE concentration at time 0, weight change, body fat content, plasma tyrosine, the sum of the remaining neutral amino acids, and the ratio of tyrosine to the remaining neutral amino acids were determined using Student's *t*-test (23). All values are expressed as mean ± SEM.

C. Results

Experiment 1. The mice which were overfed the high fat diet for three days gained weight, whereas the underfed mice lost weight. This resulted in a higher body fat content in the overfed mice (Table II-2). Since the aim of this experiment was to investigate the effects of short-term overfeeding on brain NE as distinct from the effects of a high body fat content, this difference in weight gain and body fat became a confounding variable.

Plasma tyrosine concentration did not differ between the underfed and overfed group, but the tyrosine/NAA ratio was higher in the overfed mice (Table II-3), due to lower NAA concentrations in the overfed mice.

Brain NE concentration was lower in the overfed than in the underfed mice (Table II-4). Although NE accumulation was determined over 40 min (Figure II-2), comparisons of NE synthesis rate were made by examining slopes of the lines for underfed and overfed mice, where accumulation was clearly linear as determined in the accumulation experiment (Figures II-3, II-4). Thus, in this experiment slopes of the lines between 0 and 20 min after monamine

oxidase inhibition were compared. The rate of NE synthesis in the hypothalamus was not significantly different between the underfed and overfed mice (Figure II-2, Table II-4). In the rest of the brain, the rate of NE synthesis did not increase (Figure II-2, Table II-4).

Experiment 1
Table II-2.
Effect of short-term (3d) underfeeding and overfeeding on change in weight and body fat content

Group	Change in weight[1] g	Body fat content[2] %
Underfeeding	-1.2 ± 0.2 [3a]	15.3 ± 0.7 [a]
Overfeeding	1.8 ± 0.2 [b]	22.5 ± 0.8 [b]

1 Values are means ± SEM, n = 12 - 17
2 n = 8 per group
3 Means in columns with different subscripts are significantly different ($P < 0.001$)

Experiment 1
Table II-3.
Effect of short-term (3d) underfeeding and overfeeding on plasma tyrosine and tyrosine/NAA [2]

Group	Tyrosine *µmol/dl*	NAA *µmol/dl*	Tyrosine/NAA
Underfeeding	13.0 ± 3.6[1]	170.5 ± 32.2[3a]	0.07 ± .01[a]
Overfeeding	12.0 ± 2.1	98.1 ± 13.2[b]	0.12 ± .01[b]

1 Values are means ± SEM, n = 8
2 NAA = valine, leucine, isoleucine, phenylalanine, tryptophan
3 Means in columns with different subscripts are significantly different ($P < 0.05$)

Experiment 1
Table II-4
Effect of short-term (3d) underfeeding and overfeeding on NE turnover in mouse brain

Group	Hypothalamus			Rest of the Brain		
	[NEo][1] *nmol/g*	Turnover time (T)[2] *h*	Rate of synthesis (K)[3] *nmol/g/h* ±SEK	[NEo] *nmol/g*	Turnover time (T) *h*	Rate of synthesis (K) *nmol/g/h* ±SEK
Underfeeding	7.46 ± 0.79[4a]	3.67	2.03 ± 2.77	0.47 ± 0.02	- 5.27	- 0.09 ± 0.01
Overfeeding	6.05 ± 0.50[b]	1.53	3.96 ± 1.62	0.49 ± 0.02	- 4.36	- 0.11 ± 0.02

1 Values are means ± SEM, n = 6. Rate of synthesis calculated from 6 mice per time point.
2 Time required for NE flow through the pool to equal pool size is equivalent to the time when [NEo] is doubled. From the equation of the line $y = a + bx$; $2a = a + bx$; $a = bx$; $T = a/b$ or $T = [NEo]/K$ *
3 $K = k[NEo]$ where $k = 1/T$
4 Means in columns with different subscripts are significantly different ($P < 0.05$).
* Values are derived from data shown in Figure II-2; i.e., $y = 7.46 + 2.03x$; $7.46 = 2.03x$; x or $T = 7.46/2.03$; $T = 3.67$

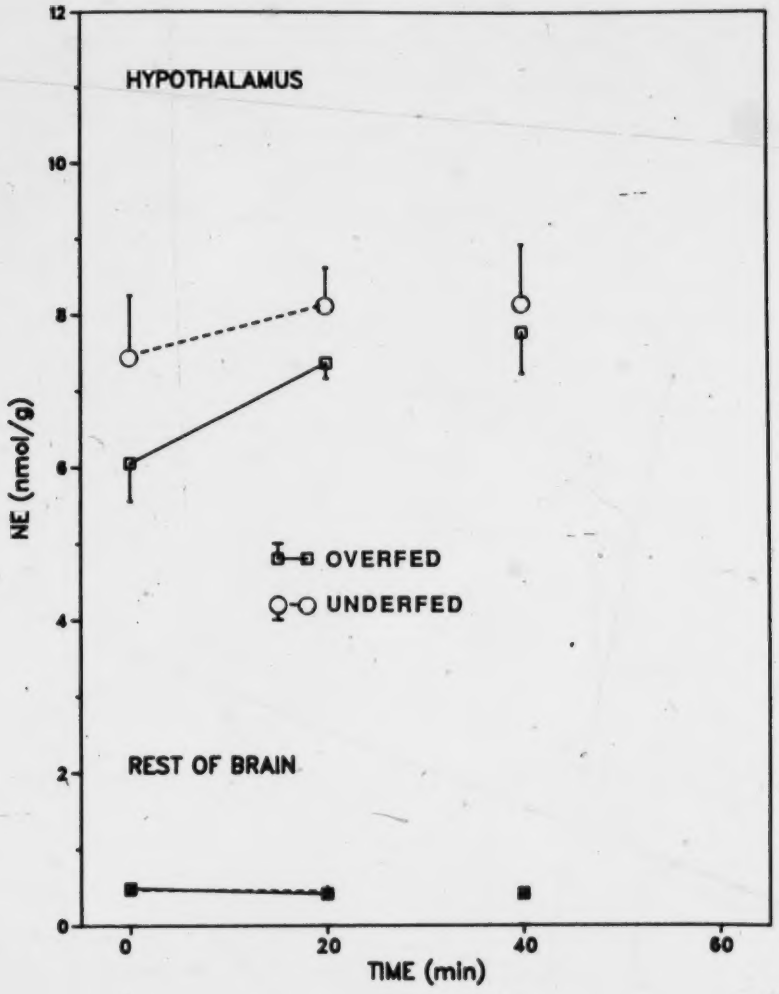

Figure II-2. Accumulation of NE in the hypothalamus and the rest of the brain after administration of pargyline and clorgyline in acutely (3d) underfed and overfed mice (Exp. 1). Each point represents mean ± SEM of 6 mice. Equations for least squares fit for the hypothalamus were $y = 7.46 + 2.03x$, $SE_b = 2.77$, $r = 0.23$, and $y = 6.05 + 3.96x$, $SE_b = 1.62$, $r = 0.61$, and for the rest of the brain were $y = 0.47 - 0.09x$, $SE_b = 0.02$, $r = 0.25$ and $y = 0.49 - 0.11x$, $SE_b = 0.02$, $r = 0.40$ in underfed and overfed mice respectively.

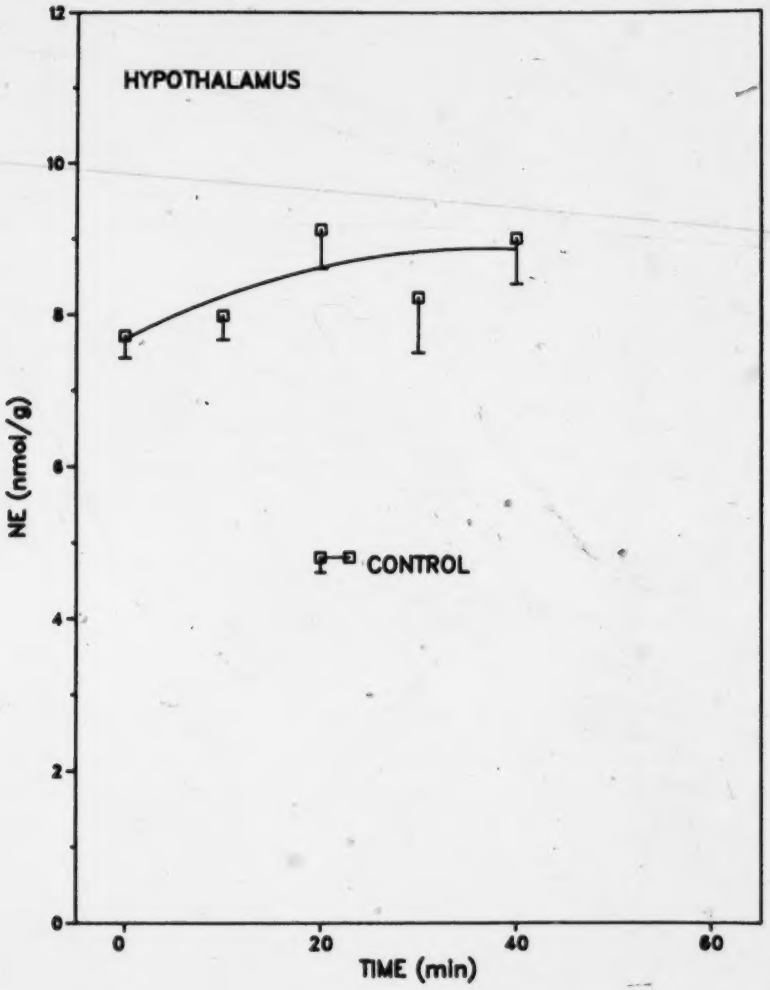

Figure II-3 Accumulation of NE in the hypothalamus after administration of pargyline and clorgyline measured at 0, 10, 20, 30 and 40 min after one week adaptation to a high fat diet to determine the time course for the best linear fit of NE accumulation. Each point represents the mean ± SEM of 3-6 mice. The equation for the polynomial fit is $y = -0.894\ (10^{-3})\ x^2 + 0.651\ (10^{-1})\ x + 7.675$, $SE_p = 1.185$.

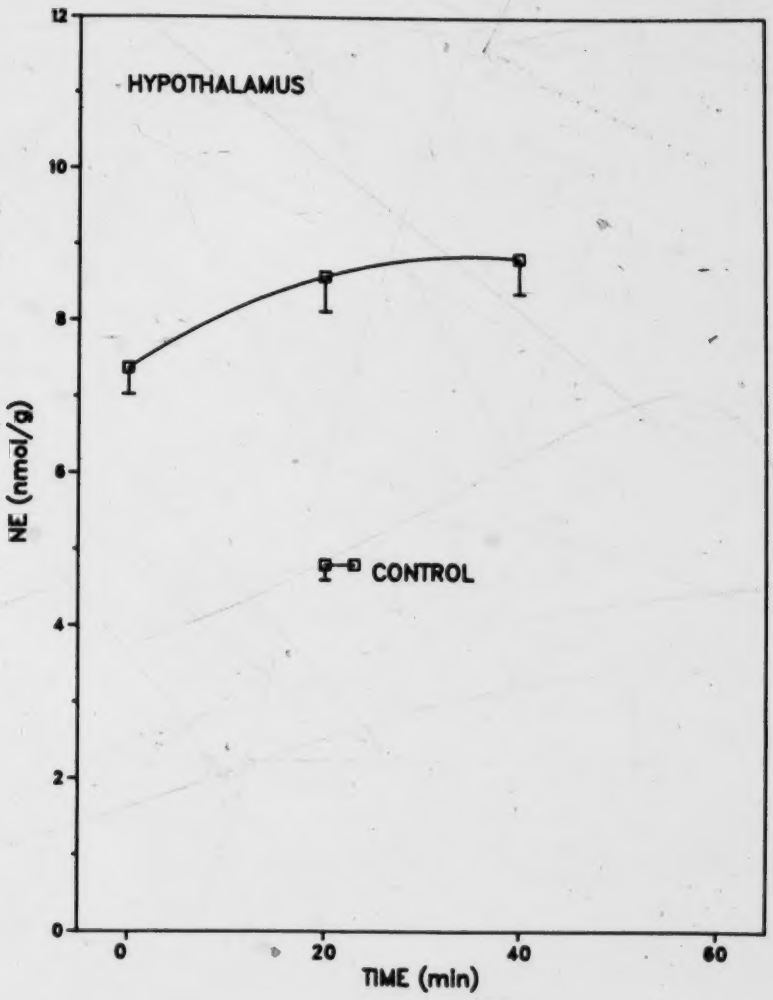

Figure II-4. Accumulation of NE in the hypothalamus after administration of pargyline and clorgyline measured at 0, 20, and 40 min after one week of adaptation to a high fat diet. The data of the two NE accumulation experiments were pooled to determine the time course where accumulation was most linear. Each point represents the mean ± SEM of 11 mice. The equation for the polynomial fit is $y = -1.209\ (10^{-3})\ x^2 + .8463\ (10^{-1})\ x + 7.375$, $SE_p = 1.305$

Experiment 2. Change in weight did not differ between the mice underfed or overfed the high fat diet for one day only (+0.3 ± 0.1 g vs. +0.6 ± 0.1 g in the underfed and overfed mice, respectively). Thus, the confounding effect of alterations in body fat content with overfeeding that occured with the three day experiment was eliminated.

Plasma tyrosine concentration and the tyrosine/NAA ratio were higher in the overfed than in the underfed mice. This result was due to the higher plasma tyrosine and lower NAA concentration in the overfed mice (Table II-5). Plasma tyrosine and the tyrosine/NAA ratio did not appear to influence NE synthesis rate, and therefore NAA analysis was not conducted in subsequent experiments.

NE concentration (Table II-6) and the rate of synthesis (Figure II-5, Table II-6) did not differ between underfed and overfed mice in the hypothalamus or in the rest of the brain.

Experiment 2
Table II-5.
Effect of short-term (1d) underfeeding and overfeeding on plasma tyrosine and tyrosine/NAA [2]

Group	Tyrosine *µmol/dl*	NAA *µmol/dl*	Tyrosine/NAA
Underfeeding	8.3 ± 2.7[1a]	131.8 ± 40.5[3a]	.06 ± .01[a]
Overfeeding	12.7 ± 2.1[b]	94.4 ± 13.9[b]	.14 ± .01[b]

[1] Values are means ± SEM, n = 7 to 9
[2] NAA = valine, leucine, isoleucine, phenylalanine, tryptophan.
[3] Means in columns with different subscripts are significantly different ($P < 0.05$).

Experiment 2
Table II-6
Effect of short-term (1d) underfeeding and overfeeding of NE turnover in mouse brain

Group	Hypothalamus			Rest of the Brain		
	$[NE_o]$[1] *nmol/g*	Turnover time (T)[2] *h*	Rate of synthesis (K)[3] *nmol/g/h* ±SEK	$[NE_o]$ *nmol/g*	Turnover time (T) *h*	Rate of synthesis (K) *nmol/g/h* ±SEK
Underfeeding	8.23 ± 0.28	2.09	3.95 ± 1.21	0.60 ± 0.02	- 2.34	- 0.26 ± 0.08
Overfeeding	8.06 ± 0.49	1.50	5.38 ± 1.70	0.59 ± 0.02	- 2.89	- 0.20 ± 0.07

[1] Values are means ± SEM, n = 6. Rate of synthesis calculated from 6 mice per time point.
[2] Time required for NE flow through the pool to equal pool size is equivalent to the time when $[NE_o]$ is doubled. From the equation of the line $y = a + bx$; $2a = a + bx$; $a = bx$; $T = a/b$ or $T = [NE_o]/K$ *
[3] $K = k[NE_o]$ where $k = 1/T$
* Values are derived from data shown in Figure II-5.

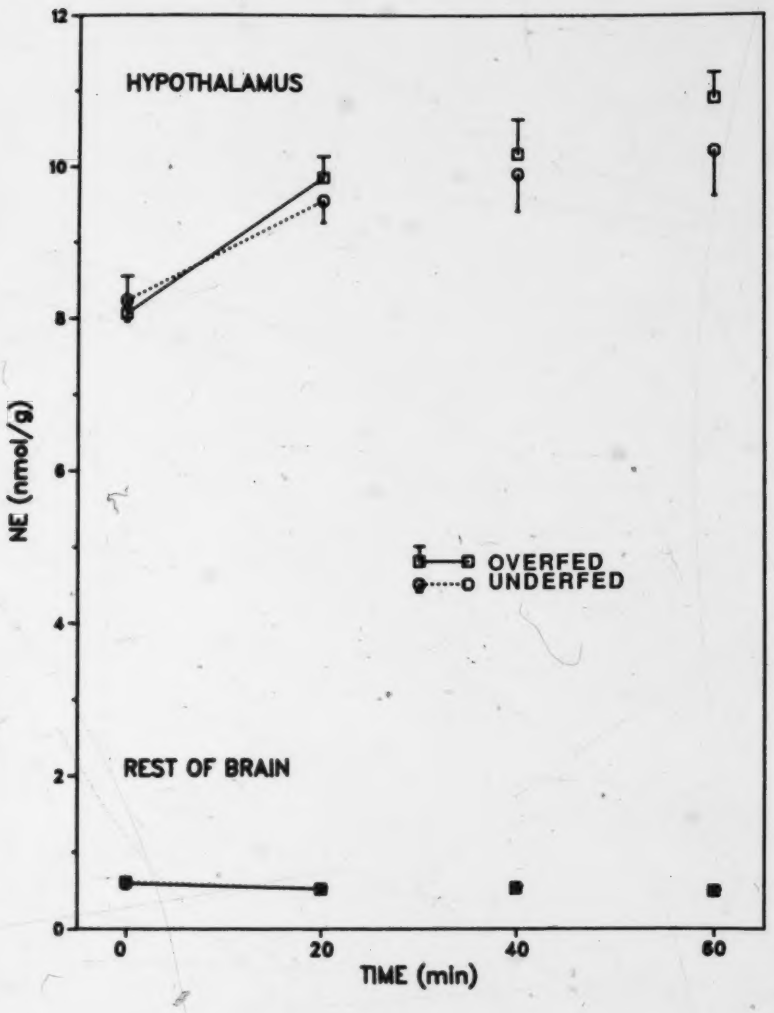

Figure II-5. Accumulation of NE in the hypothalamus and the rest of the brain after administration of pargyline and clorgyline in acutely (1d) underfed and overfed mice (Exp. 2). Each point represents mean ± SEM of 6 mice. Equations for least squares fit for the hypothalamus were $y = 8.23 + 3.95x$, $SE_b = 1.21$, $r = 0.72$, and $y = 8.06 + 5.38x$, $SE_b = 1.70$, $r = 0.71$, and for the rest of the brain were $y = 0.60 - 0.26x$, $SE_b = 0.08$, $r = 0.70$ and $y = 0.59 - 0.20x$, $SE_b = 0.07$, $r = 0.67$ in underfed and overfed mice respectively.

Experiment 3. Chronic overfeeding of the high fat diet for eleven days resulted in a higher body fat content and a greater weight gain than in the underfed mice (Table II-7). The difference in body fat content is mainly attributable to weight and fat loss in the underfed group. Body fat content in chronically overfed mice did not significantly differ from that of mice acutely overfed in Exp. 1.

Brain NE concentration in the hypothalamus and the rest of the brain did not differ between the chronically underfed and overfed mice (Table II-8). Comparisons of NE synthesis rate were made by examining slopes of the lines between 0 and 40 min after NE catabolism inhibition, because the accumulation of NE was linear for at least 40 min. Rate of NE synthesis in the hypothalamus tended to be higher in the overfed group, but the slopes of the regression lines were not significantly different (Figure II-6, Table II-8). NE synthesis rate in the rest of the brain did not increase in either group; further examination of NE synthesis rate in the rest of the brain was considered irrelevant to the study.

Experiment 3
Table II-7
Effect of chronic (11d) underfeeding and overfeeding on change in weight and body fat content

Group	Change in weight[1] *g*	Body fat content[2] %
Underfeeding	-1.3 ± 0.2 [3a]	11.9 ± 0.8 [a]
Overfeeding	2.8 ± 0.3 [b]	18.7 ± 0.8 [b]

1 Values are means ± SEM, n = 21 - 24
2 n = 8 per group
3 Means in columns with different subscripts are significantly different ($P < 0.001$)

Experiment 3
Table II-8
Effect of chronic (11d) underfeeding and overfeeding of NE turnover in mouse brain

Group	Hypothalamus			Rest of the Brain		
	$[NE_o]$[1] *nmol/g*	Turnover time (T)[2] *h*	Rate of synthesis (K)[3] *nmol/g/h* ±SEK	$[NE_o]$ *nmol/g*	Turnover time (T) *h*	Rate of synthesis (K) *nmol/g/h* ±SEK
Underfeeding	7.65 ± 0.45	2.22	3.35 ± 1.20	0.60 ± 0.03	- 6.20	- 0.10 ± 0.06
Overfeeding	7.28 ± 0.19	1.39	5.25 ± 0.78	0.54 ± 0.03	- 8.77	- 0.06 ± 0.05

1 Values are means ± SEM, n = 6. Rate of synthesis calculated from 6 mice per time point.
2 Time required for NE flow through the pool to equal pool size is equivalent to the time when $[NE_o]$ is doubled. From the equation of the line $y = a + bx$; $2a = a + bx$; $a = bx$; $T = a/b$ or $T = [NE_o]/K$ *
3 $K = k[NE_o]$ where $k = 1/T$
* Values are derived from data shown in Figure II-6.

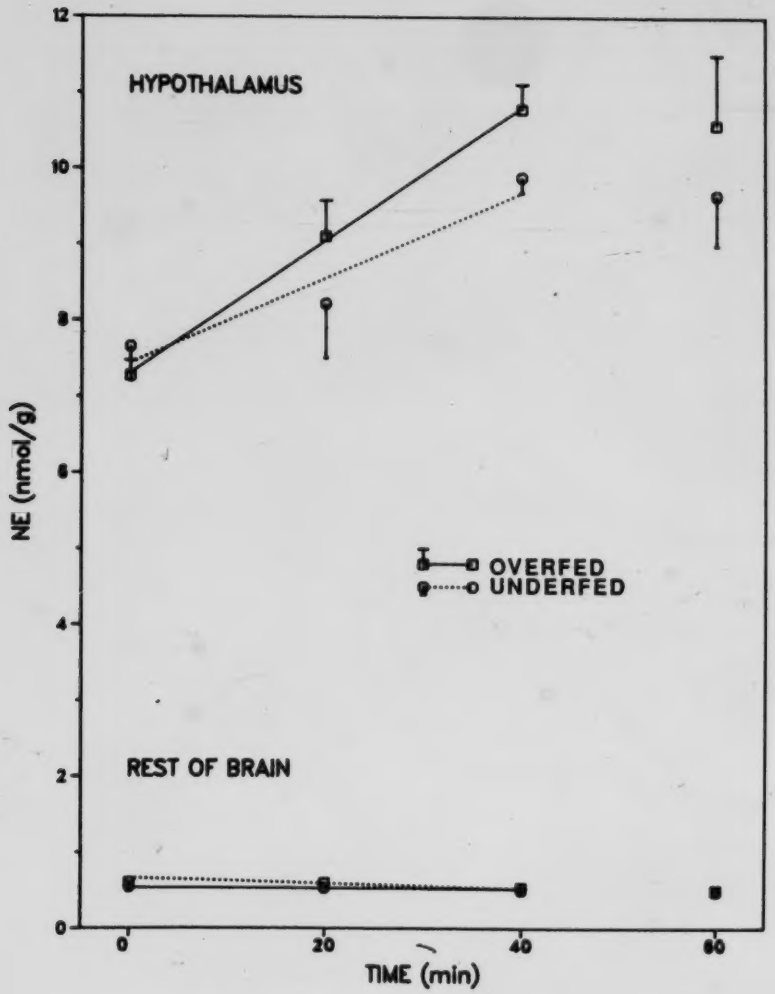

Figure II-6. Accumulation of NE in the hypothalamus and the rest of the brain after administration of pargyline and clorgyline in chronically (11d) underfed and overfed mice (Exp. 3). Each point represents mean ± SEM of 6 mice. Equations for least squares fit for the hypothalamus were $y = 7.65 + 3.35x$, $SE_b = 1.20$, $r = 0.64$, and $y = 7.28 + 5.25x$, $SE_b = 0.78$, $r = 0.89$, and for the rest of the brain were $y = 0.60 - 0.10x$, $SE_b = 0.06$, $r = 0.40$ and $y = 0.54 - 0.06x$, $SE_b = 0.05$, $r = 0.35$ in underfed and overfed mice respectively.

Experiment 4. Because the hypothalamus is comprised of several distinct nuclei, it has been suggested that the assay of the whole hypothalamus might obscure discrete variations in neurochemical parameters in smaller regions (13). Thus, NE synthesis rate in individual nuclei was investigated.

Chronic overfeeding of the high fat diet for eleven days produced a higher body fat content and a greater weight gain in the overfed mice (Table II-9), similar to Exp. 3.

Sections used for the removal of specific hypothalamic areas are presented in Table II-10. The protein content ranged from 16.98 ± 1.08 µg in the suprachiasmatic nucleus (Sch) to 40.34 ± 1.50 µg in the lateral hypothalamus (LH).

The results of the NE analysis indicated substantial differences in the NE concentrations of the various nuclei (Table II-11). The ventromedial nucleus (VMH) was the only hypothalamic area studied that showed a significant difference in NE concentration between underfed and overfed mice, with chronic overfeeding producing a 32.2% higher NE concentration.

Rate of NE synthesis did not differ between underfed and overfed mice in any of the nuclei studied (Figure II-7, Table II-11).

Experiment 4
Table II-9
Effect of chronic (11d) underfeeding and overfeeding on change in weight and body fat content

Group	Change in weight[1] g	Body fat content[2] %
Underfeeding	-1.4 ± 0.2 [3a]	11.6 ± 0.6 [a]
Overfeeding	3.2 ± 0.2 [b]	18.3 ± 0.9 [b]

1 Values are means ± SEM, n = 9
2 n = 6 per group
3 Means in columns with different subscripts are significantly different ($P < 0.05$)

Experiments 4 and 5
Table II-10
Coordinates used for the removal of specific hypothalamic nuclei from underfed, overfed ad libitum and food-deprived mice and the protein content of each nucleus

Hypothalamic area	Punches/ brain	Coordinates	Protein content[1] µg
Suprachaismatic nucleus	2	F-0.3 to F-0.9	16.98 ± 1.08
Anterior hypothalamus	6	F-0.3 to F-1.2	36.23 ± 1.58
Paraventricular nucleus	4	F-0.6 to F-1.2	28.67 ± 1.55
Ventromedial nucleus	8	F-1.2 to F-2.4	39.93 ± 1.24
Dorsomedial nucleus	6	F-1.2 to F-2.1	29.50 ± 1.18
Lateral hypothalamus	8	F-1.2 to F-2.4	40.34 ± 1.50
Arcuate nucleus	4	F-1.5 to F-2.7	19.75 ± 0.93

[1] Values are means ± SEM, n = 18-24. For the punches, all nuclei were removed using a 430 µm internal diameter needle. Coordinates are from the atlas of Slotnick and Leonard (17).

Experiment 4
Table II-11
Effect of chronic (11d) underfeeding and overfeeding on NE turnover in hypothalamic nuclei in mice

Group		[NEo][1] *pg/µg protein*	Turnover time (T)[2] *h*	Rate of synthesis (K)[3] *pg/µg protein/h* ± SEK
Suprachaismatic nucleus	U[5]	12.41 ± 0.30	1.28	9.70 ± 4.05
	O	13.01 ± 1.01	1.39	9.33 ± 2.77
Anterior hypothalamus	U	12.37 ± 1.86	1.71	7.24 ± 2.97
	O	11.69 ± 1.10	1.00	11.69 ± 2.47
Paraventricular nucleus	U	16.51 ± 2.12	4.65	3.55 ± 4.24
	O	13.24 ± 2.36	2.14	6.20 ± 4.74
Ventromedial nucleus	U	13.41 ± 1.53 [4a]	2.47	5.44 ± 2.79
	O	19.79 ± 1.47 [b]	1.49	13.29 ± 4.05
Dorsomedial nucleus	U	23.65 ± 1.99	11.00	2.15 ± 5.79
	O	25.56 ± 2.12	8.24	2.86 ± 4.99
Lateral hypothalamus	U	12.43 ± 1.13	2.54	4.90 ± 2.33
	O	12.81 ± 0.51	1.39	9.19 ± 4.08
Arcuate nucleus	U	17.08 ± 2.55	1.72	9.91 ± 6.16
	O	16.98 ± 2.28	1.71	9.93 ± 5.82

1 Values are means ± SEM, n = 6. Rate of synthesis calculated from 6 mice per time point.
2 Time required for NE flow through the pool to equal pool size is equivalent to the time when [NEo] is doubled. From the equation of the line y = a + bx; 2a = a + bx; a = bx; T = a/b or T = [NEo]/K
3 K = k[NEo] where k = I/T
4 Means in the group with different subscripts are significantly different ($P < 0.05$)
5 U = underfed; O = overfed
* Values are derived from data shown in Figure II-7; i.e., y = 12.41 + 9.70x; 12.41 = 9.70x; x or T = 12.41/9.70; T = 1.28.

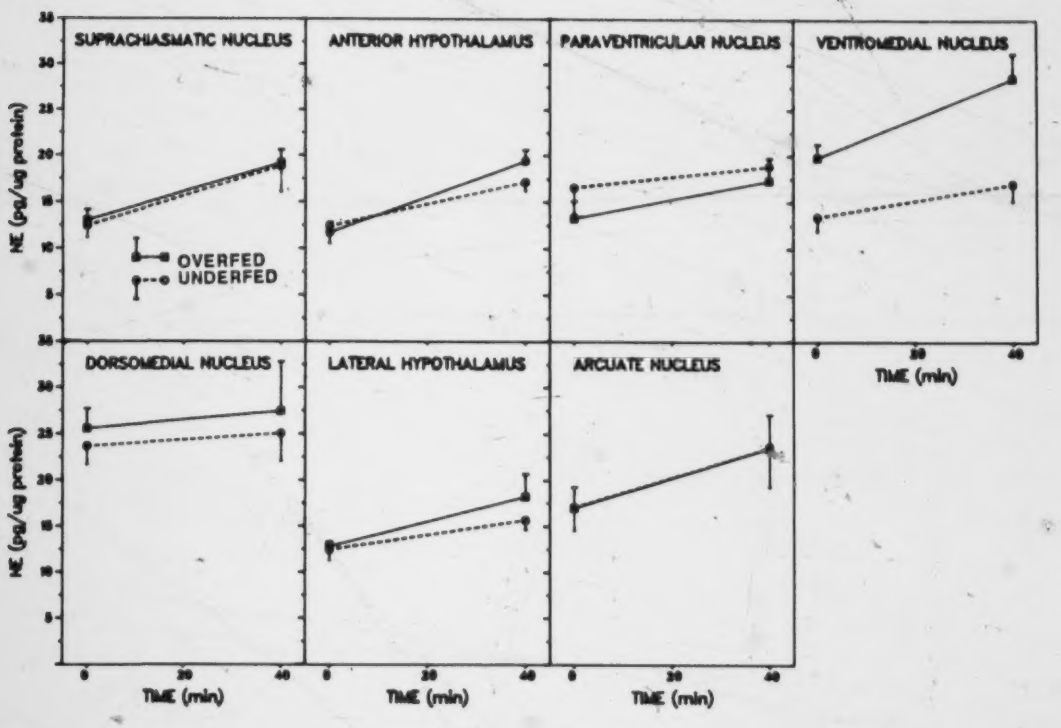

Figure II-7. Accumulation of NE in specific hypothalamic nuclei after administration of pargyline and clorgyline in chronically (11d) underfed (U) and overfed (O) mice (Exp. 4). Each point represents mean ± SEM of 4 to 5 mice. Equations for least squares fit were as follows:

SCH	U	$y = 12.41 + 9.70x$,	$SE_b = 4.05$
	O	$y = 13.01 + 9.33x$,	$SE_b = 2.77$
ANT	U	$y = 12.37 + 7.24x$,	$SE_b = 2.97$
	O	$y = 11.69 + 11.69x$,	$SE_b = 2.47$
PVN	U	$y = 16.51 + 3.55x$,	$SE_b = 4.24$
	O	$y = 13.24 + 6.20x$,	$SE_b = 4.74$
VMH	U	$y = 13.41 + 5.44x$,	$SE_b = 2.79$
	O	$y = 19.79 + 13.29x$,	$SE_b = 4.05$
DM	U	$y = 23.65 + 2.15x$,	$SE_b = 5.79$
	O	$y = 25.56 + 2.89x$,	$SE_b = 4.99$
LH	U	$y = 12.43 + 4.90x$,	$SE_b = 2.33$
	O	$y = 12.81 + 9.19x$,	$SE_b = 4.08$
ARC	U	$y = 17.08 + 9.91x$,	$SE_b = 6.16$
	O	$y = 16.98 + 9.93x$,	$SE_b = 5.82$

Experiment 5. Mice were ad libitum fed or deprived of food for 24 h to assess potential alterations in NE synthesis rate within specific hypothalamic sites in relation to the feeding status of the mice.

Sections used for the removal of specific hypothalamic areas are presented in Table II-10. Concentration of NE between ad libitum and food-deprived mice did not differ significantly in any of the hypothalamic areas studied (Table II-12).

In the food-deprived mice, rate of NE synthesis in the paraventricular nucleus (PVN) and at no other site was five-fold higher than in the fed mice (Figure II-8, Table II-12).

Experiment 5
Table II-12
Effect of 24h food deprivation on NE turnover in hypothalamic nuclei in mice

Group		[NEo][1] *pg/µg protein*	Turnover time (T)[2] *h*	Rate of synthesis (K)[3] *pg/µg protein/h* ± SEK
Suprachaismatic nucleus	D[5]	15.68 ± 1.56	3.79	4.14 ± 4.93
	F	13.34 ± 1.90	1.14	11.68 ± 3.75
Anterior hypothalamus	D	12.20 ± 0.92	0.99	12.36 ± 4.49
	F	12.26 ± 0.96	0.93	13.17 ± 2.76
Paraventricular nucleus	D	19.41 ± 2.12	0.68	28.47 ± 6.76 [4a]
	F	20.54 ± 2.36	3.55	5.79 ± 3.93 [b]
Ventromedial nucleus	D	12.81 ± 1.83	0.86	14.85 ± 4.69
	F	13.55 ± 1.01	0.69	19.53 ± 3.31
Dorsomedial nucleus	D	27.12 ± 2.41	3.04	8.93 ± 3.90
	F	28.99 ± 2.24	7.14	4.06 ± 3.75
Lateral hypothalamus	D	11.07 ± 1.82	3.57	3.10 ± 4.30
	F	9.53 ± 0.64	0.88	10.87 ± 1.71
Arcuate nucleus	D	14.67 ± 1.93	1.24	11.84 ± 5.53
	F	15.05 ± 1.64	1.40	10.73 ± 3.86

1 Values are means ± SEM, n = 6. Rate of synthesis calculated from 6 mice per time point.
2 Time required for NE flow through the pool to equal pool size is equivalent to the time when [NEo] is doubled. From the equation of the line $y = a + bx$; $2a = a + bx$; $a = bx$; $T = a/b$ or $T = [NEo]/K$ *
3 $K = k[NEo]$ where $k = 1/T$
4 Means in the group with different subscripts are significantly different ($P < 0.05$)
5 D = food deprived; F = fed.
* Values are derived from data shown in Figure II-7.

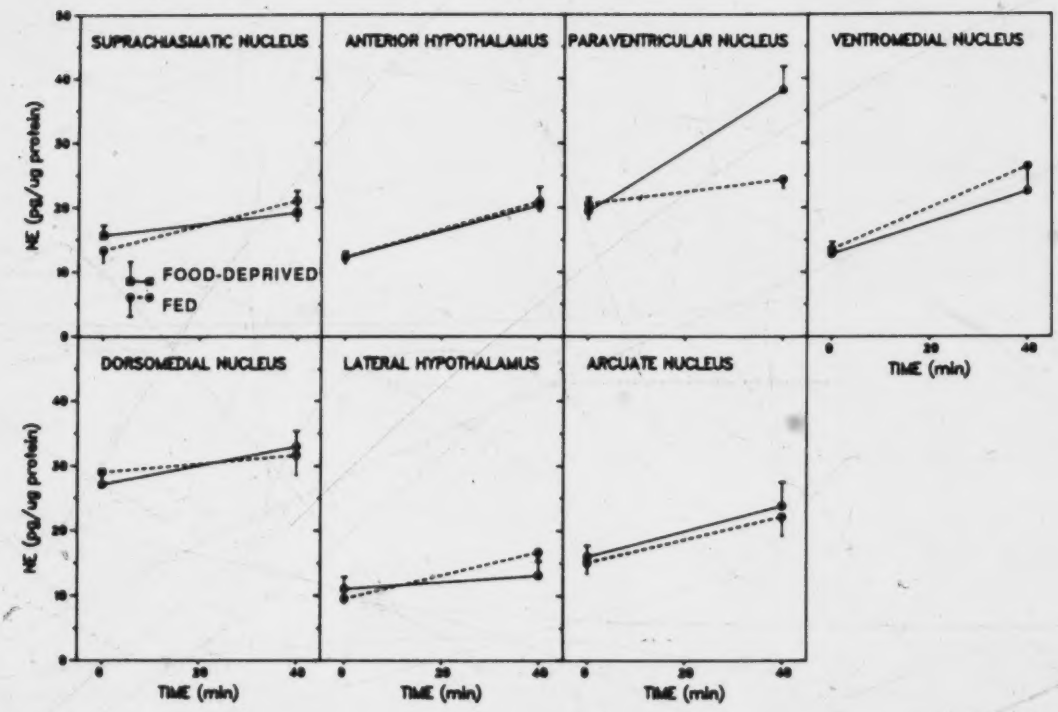

Figure II-8. Accumulation of NE in specific hypothalamic nuclei after administration of pargyline and clorgyline in 24h food-deprived (D) and fed (F) mice (Exp. 5). Each point represents mean ± SEM of 6 mice. Equations for least squares fit were as follows:

SCH	D	$y = 15.68 + 4.14x$,	$SE_b = 4.93$
	F	$y = 13.34 + 11.68x$,	$SE_b = 3.75$
ANT	D	$y = 12.20 + 12.36x$,	$SE_b = 4.49$
	F	$y = 12.26 + 13.17x$,	$SE_b = 2.76$
PVN	D	$y = 19.41 + 28.47x$,	$SE_b = 6.76$
	F	$y = 20.54 + 5.79x$,	$SE_b = 3.93$
VMH	D	$y = 12.81 + 14.85x$,	$SE_b = 4.69$
	F	$y = 13.55 + 19.53x$,	$SE_b = 3.31$
DM	D	$y = 27.12 + 8.93x$,	$SE_b = 3.90$
	F	$y = 28.99 + 4.06x$,	$SE_b = 3.75$
LH	D	$y = 11.07 + 3.10x$,	$SE_b = 4.30$
	F	$y = 09.53 + 10.87x$,	$SE_b = 1.71$
ARC	D	$y = 14.67 + 11.84x$,	$SE_b = 5.53$
	F	$y = 15.05 + 10.73x$,	$SE_b = 3.86$

D. Discussion and Conclusions

The results of this study indicate that hypothalamic norepinephrine (NE) synthesis rate is more related to short-term food intake regulation than to the thermic or thermogenic effects of overeating. In addition, the effects of food intake and deprivation appear to be site-specific in the hypothalamus. Specifically, mice deprived of food for 24h had a five-fold higher NE synthesis rate in the paraventricular nucleus (PVN) than in the fed mice. Altering the body fat content of normal mice by chronically underfeeding or overfeeding did not alter brain NE synthesis rate, neither in the whole hypothalamus nor in hypothalamic nuclei of fed mice.

These results are important for several reasons. First, they separate the effects of relative overfeeding and underfeeding from food deprivation on NE synthesis rate. Second, they help to clarify the causal nature of the reduced brain NE synthesis rate seen in the obese genotype of this strain of mouse. These findings do not support the idea that decreased brain metabolism in young genetically obese (*ob/ob*) mice (24) is a consequence of their elevated body fat content, or a consequence of overeating, but rather that the abnormality seen in this genetically obese animal model may be causal of their obesity, or at least an association with the obese phenotype.

In relation to relative overfeeding and underfeeding, the rate of NE synthesis in the hypothalamus was not significantly different between underfed and overfed mice in either the acute (1d, 3d) or chronic (11d) feeding studies. NE synthesis rate was measured during the thermic effect of the previous meal, a time when the thermic effect of underfeeding and overfeeding should be maximally apparent. All animals in the acute and chronic under- and over-feeding studies were in the fed state, having each received a meal 20 min prior to the time zero injection and killing. Likewise in the hypothalamic nuclei of the

chronically underfed or overfed mice, NE synthesis rate did not differ between treatment groups. These studies thus indicate that NE synthesis rate in the whole hypothalamus or in specific hypothalamic nuclei is not differentially affected during the thermic effect of the previous meal, in either acutely or chronically underfed or overfed mice. The acute effects of food, however, have been reported to peak at 30 min in humans and to last for 2 h (25). In mice fed 25% of ad libitum intake after a 24h fast, oxygen consumption increased by 17% at 20 min after presentation of the meal (J.L. Johnston and R. Graval, unpublished observation).

In the 24h food-deprived mice in the present study, the rate of NE synthesis in the PVN and at no other site was five times higher than in the fed mice. This is the first report of increased NE synthesis rate in the PVN of fasted mice. This observation supports pharmacological evidence for a physiological role for PVN-NE as a stimulus for the ingestion of food. Specifically, activity of the α-adrenergic receptor is lower in the PVN of rats deprived of food for 48h than in fed rats (26.). The down-regulation of α-adrenergic receptors in the PVN has been attributed to an increase in NE synthesis rate in this nucleus, which suggests a potential role for the PVN-NE system in mediating deprivation-induced eating. Hyperphagia and body weight gain are observed following NE administration into the PVN, suggesting that endogenous NE in this nucleus may act to enhance food intake by inhibiting a specific PVN-controlled satiety function (27). A similar set of phenomena (increase in food intake and body weight gain) occurs following electrolytic lesions in the PVN in rats maintained on ad libitum feeding (28), implicating the PVN in the control of normal feeding behavior. Jhanwar-Uniyal *et al.* (1987), using α-methyl-para-tyrosine to deplete endogenous NE 3h prior to decapitation and estimate NE turnover, found an increase in NE turnover exclusively in the PVN following

48h food deprivation (29). These data suggest that hypothalamic noradrenergic activity, specifically in the PVN, varies as a function of food deprivation and feeding.

In the present study, altering body fat content of normal mice did not alter brain NE synthesis rate. Specifically, no significant differences in NE synthesis rate were associated with normal or low body fat content of the overfed or underfed mice, respectively. The difference in body fat content in the present study is mainly attributable to weight and fat loss in the underfed group. Body fat content in chronically overfed mice did not differ from that of mice which were acutely overfed, possibly because although ad libitum quantity was fed, the food was given once daily and therefore the mice were not truly "ad libitum" fed. In the chronic overfeeding experiment (Exp. 4), the concentration of NE in the ventromedial nucleus (VMH) was significantly higher in the overfed than in the underfed animals, but no difference in NE synthesis rate was observed. Although there was no difference in NE synthesis rate at the level of the whole hypothalamus or in specific nuclei, the increased concentration of NE in the VMH of chronically overfed mice suggests NE metabolism may be altered and warrants further investigation.

NE synthesis rate in the present study was determined from the rate of accumulation of NE after inhibition of NE catabolism by the monamine oxidase (MAO) inhibitors, pargyline and clorgyline. The use of NE accumulation as an indicator of brain NE synthesis relies on the assumption that pargyline and clorgyline completely block MAO activity. Pargyline at a high dose (75 mg/kg), as used in this study, is an irreversible inhibitor of both MAO-A and MAO-B activity, resulting in a 98% inhibition of MAO activity within 5 min of injection, lasting 3 h in rats (30). A 93% inhibition of brain MAO-A activity occurs 1 min after a low dose (10 mg/kg) of clorgyline in mice and lasts 1 h (31). Although

MAO activity was not measured in the present study, it is assumed that the combination of pargyline and clorgyline blocked MAO activities similarly in these mice. It is possible that the rate of synthesis determined for NE as used in this study may slightly underestimate the true rate of synthesis, because accumulation of NE may diminish its own synthesis by end-product inhibition of tyrosine hydroxylase (32). This may have occurred in the acute feeding studies after 20 min of injection, because the rate of accumulation of NE was not linear beyond 20 min. However, in these studies, the rate of NE synthesis was determined from the accumulation over the first 20 min where the slope was most linear. In the chronic feeding experiments, the NE accumulation was linear for at least 40 min, suggesting the possibility of developmental changes in NE metabolism. It is possible, during the 11 day period of over- and underfeeding, that some maturation of NE synthetic or catabolic enzymes occured. This possibility is supported by the observation in 12-day-old rats that NE concentration in the hypothalamus is very low (33.6 nmol/g) and a gradual increase is observed during the postnatal growth of the rat, resulting in a NE concentration of 99.0 nmol/g at 75 days of age (33), suggesting an alteration in NE metabolism. However, interpretation of changes in brain NE concentration with age must be made with caution; the alteration of NE content may suggest changes in synthesis rate or catabolism or both, but changes in NE concentration are not consistently associated with differences in turnover.

In the chronic feeding experiments, the NE accumulation was linear for at least 40 min. The curvilinear accumulation after 20 min in the acute feeding experiments, and after 40 min in the chronic feeding experiments was somewhat unexpected due to the previously observed 3h duration of MAO inhibition in the whole brains of rats (30). It appears that the rate of accumulation is much faster in the hypothalamus than it is in the whole brain,

and hence feedback inhibition by this raplidy accumulating NE may be apparent much sooner in the hypothalamus than in the whole brain. Li *et al.* (1984) have shown a linear accumulation of NE in whole mouse brain for 30 min after a combination of monamine oxidase inhibition by pargyline and clorgyline, plus catechol-O-methyltransferase (COMT) inhibition by tropolone. This resulted in an NE synthesis rate of 944 pmol/g/h in whole brain over the linear 30 min, with the accumulation becoming curvilinear after 1 h (31). Johnston *et al.* (1986) observed a linear NE accumulation until 0.75 h using pargyline to block NE catabolism, resulting in an NE synthesis rate of 1046 pmol/g/h in whole mouse brain (24). Our values ranged from 2030 pmol/g/h to 5380 pmol/g/h (avg. 3987 pmol/g/h) in the whole hypothalamus, measured over 20 min in the acute feeding studies and over 40 min in the chronic feeding study. In the present study, NE synthesis rate in the rest of the brain did not increase. This suggests that very little noradrenergic activity is present in the rest of the brain and that noradrenergic activity is centered in the hypothalamus or in specific sites in the hypothalamus.

Lorden *et al.* (1976) reported a lower NE turnover in the hypothalamus of lean mice (2074 pmol/g/h) than that reported in our study, by measuring the rate of decline of NE over 3h following α-methyl-para-tyrosine administration (34). A more rapid accumulation of NE observed in our study may reflect a rapidly turning over pool of NE. The measurement of a NE pool with rapid turnover may be missed by examining the decline in NE concentration hourly for 3h after synthesis inhibition by α-methyl-para-tyrosine administration. The existence of more than one pool of NE in the brain is a matter of some debate (35). However, a two-compartment model of storage of catecholamines in the brain has been proposed to account for the rapid release of NE after nerve stimulation, reflecting a functional pool of newly synthesized catecholamines,

and a much slower release of a larger storage pool (36). Short-term increases in NE concentration can be more reliably detected by measuring the accumulation of NE after MAO inhibition. Inhibition of NE catabolism by MAO inhibitors is one of the most sensitive methods of assessing NE turnover, if measurements are made where NE accumulation is clearly linear (31).

NE concentration in the whole hypothalamus at time zero of both underfed and overfed mice in the acute and chronic feeding studies averaged 7.46 ± 0.77 nmol/g and was in close agreement with other reported values of 9.51 ± 0.33 nmol/g observed by Lorden *et al.* (1976) (34). The concentrations of NE in the specific hypothalamic nuclei found in the present study were also similar to those reported by Oltmans (1983) (37). Differences in NE concentration in the whole hypothalamus did not appear to parallel differences in NE synthesis rate, because NE concentration was significantly higher in mice underfed for 3d than in their overfed counterparts, while the rate of NE synthesis tended to be higher in the overfed group, although the slopes of the regression lines were not significantly different. This observation of a higher NE concentration in underfed than in overfed mice is similar to that in adult male rats semistarved for three weeks, in which NE concentration was significantly higher in the mediobasal hypothalamus, and 3-methoxy-4-hydroxyphenylglycol (MHPG) concentration was lower (suggesting lower NE turnover) than in controls when measured serially at 8 time points over a 24h period (38).

The NE concentration in the VMH was 32.2% higher in chronically overfed mice, but the NE synthesis rate was not significantly different between the underfed and overfed mice. Levin *et al.* found no difference in the NE concentration or turnover in the VMH of rats which were chronically overfed for three months, compared to ad libitum fed controls, but observed a higher

turnover of NE in the PVN and the dorsomedial nucleus of the overfed rats (40). It is difficult to compare these results to the present study, due to the differences in the method of assessing turnover (endogenous NE levels x fractional turnover, measured over 3h after α-methyl-para-tyrosine administration), the species, the type of diets fed, the duration of overfeeding, and the time of killing. Levin *et al.* (1986) fed control animals a 4.5% fat diet, ad libitum, whereas the overfed animals received a 16% fat diet, producing a 70% greater weight gain in the overfed rats (40). The study did not indicate whether the rats were killed at the end of the dark or light period, whereas the mice in our study were killed at the end of the dark period, in the fed state. Since prior food deprivation affects PVN-NE synthesis rate, a difference in study design may explain this discrepancy.

In our study, the chronically overfed mice did not differ in body fat content from the acutely overfed mice. Hence, the resulting intake did not produce obese mice, but clearly resulted in the overfed mice being much fatter than the underfed mice. A longer experiment allowing a more pronounced gain in body fat content of the overfed mice would be desirable to test if specific brain areas such as the VMH are a stimulus for diet-induced thermogenesis (DIT) in chronically overfed mice. The role of NE in the thermic effect of eating, however, should be apparent in this study, both acutely (after 1d and 3d of overfeeding) and chronically (after 11d of overfeeding), because mice in all of these cases were given a meal 20 min prior to killing at the end of the dark period.

Because tyrosine availability to the brain has been proposed as influencing brain NE metabolism (41), the ratio of tyrosine to neutral amino acids (NAA) in plasma was examined in the underfed and overfed mice to determine if brain tyrosine availability influenced hypothalamic NE synthesis

rate. Although NE synthesis rate tended to be higher in the overfed mice, the difference from underfed mice was not significant and was not paralleled by the two-fold higher plasma tyrosine/NAA ratio in the overfed mice, nor presumably in the brain tyrosine concentration. It has been proposed that, in rats, the major factor determining the transport of a neutral amino acid from blood capilliaries into brain neurons is the concentration of that amino acid relative to the concentrations of the amino acids with which it competes for uptake across the blood brain barrier (13). Based on evidence that a low plasma tyrosine/NAA ratio is associated with a low uptake of tyrosine to the brain (42), it has been postulated that altered NE metabolism could be attributed to changes in the availability of the NE precursor in the brain. In the present study, the plasma ratio of tyrosine to NAA was lower in the underfed than overfed mice in both short-term experiments, with the difference being more pronounced in the shortest term (1d) overfeeding experiment. The hypothalamic synthesis rate, however, did not differ between under- and overfed mice in these two experiments.

The difference in the plasma tyrosine/NAA ratio between under- and overfed mice was mainly attributable to lower NAA concentrations in the overfed mice. The sum of plasma concentrations of the NAAs was higher in underfed than in overfed mice. This was an unexpected observation, because the plasma NAA concentrations have been reported to reflect habitual protein intake (13) and the quantity of protein was constant across both groups. It is possible that the higher NAA concentrations in the underfed mice and the lower NAA concentrations in the overfed mice may be due to the different energy intakes on insulin release (43).

The findings that plasma tyrosine and the tryosine/NAA ratio was unrelated to NE synthesis has been supported by others. Johnston *et al.*

(1986) fed the same strain of mice a 20% protein diet (1.1% tyrosine) or a 20% diet supplemented with 4% tyrosine, and although brain tyrosine concentration was doubled, it had no effect on brain NE synthesis rate (24). Thus, tyrosine availability does not appear to be the determining factor for NE synthesis rate for these mice.

It is concluded that hypothalamic NE synthesis rate is more related to short-term food intake regulation than to the thermic effects of eating. The effects of food intake and deprivation appear to be site-specific in the hypothalamus, with NE synthesis rate being higher in the PVN of 24h food-deprived mice than in fed mice. The higher NE synthesis rate in the PVN of the 24h food-deprived mice is consistent with previous pharmacological evidence that an increase in noradrenergic activity in the PVN may be a stimulus for food ingestion. Altering the body fat content of normal mice by chronic over- and underfeeding did not alter brain NE synthesis rate in the whole hypothalamus or in specific hypothalamic nuclei of fed mice. This finding indicates that the reduced brain NE synthesis rate previously seen in the obese genotype of this strain of mice may cause their obesity, or at least an association with the obese phenotype and not a consequence of their elevated body fat. The increased concentration of NE in the VMH in the chronically overfed mice may suggest altered NE metabolism and warrants further investigation.

E. References

1. Van Itallie, T.B., Gale, S.K., and Kissileff, H.R. (1978). Control of food intake in the regulation of depot fat: an overview. In: Advances in Modern Nutrition (Katzen, H.M., Mahler, R.J., eds.) pp. 427-492, Hemisphere Press, London.

2. Rossi III, J., Zolovick, A.J., Davis, R.F., and Pansksepp, J. (1982) The role of norepinephrine in feeding behavior. Neurosci. Biobehav. Rev. *6*, 195-204.

3. Morley, J.E., Gosnell, B.A., Krahn, D.D., Mitchell, J.E. and Levine, A.S. (1985) Clinical implications of neurochemical mechanisms of appetite regulation. Psychopharm. Bull. *21*, 400-405.

4. Glick, S.D., Waters, D.H. and Milloy, S. (1973) Depletion of hypothalamic norepinephrine by food deprivation and interaction with D-amphetamine. Res. Commun. Chem. Pathol. Pharmacol. *6*, 775-778.

5. Stachowiak, M., Bialowas, J. and Jurkowski, M. (1978) Catecholamines in some hypothalamic and telencephalic nuclei of food-deprived rats. Acta Neurobiol. Exp. *38*, 157-165.

6. Loullis, C.C., Felten, D.L. and Shea, P.A. (1979) HPLC determinations of biogenic amines in discrete brain areas in food-deprived rats. Pharmcol. Biochem. Behav. *11*, 89-93.

7. Friedman, E., Starr, N. and Gershon, S. (1973) Catecholamine synthesis and the regulation of food intake in the rat. Life Sci. *12*, 317-326.

8. Pirke, K.M. and Spyra, B. (1980) Catecholamine turnover in the brain and the regulation of lutenizing hormone and corticosterone in starved male rats. Acta Endrocrinol. *100*, 168-172.

9. Shimazu, T. and Takahashi, A. (1980) Stimulation of hypothalamic nuclei has differential effects on lipid synthesis in brown and white adipose. Nature *284*, 62-63.

10. Barbosa, M.C. and Migliorini, R.H. (1982) Free fatty acid mobilization in rats following intracerebroventricular norepinephrine. Am. J. Physiol. *242*, E248-E252.

11. Levin, B.E., Triscari, J. and Sullivan, A. (1985) The effect of diet and chronic obesity on brain catecholamine turnover in the rat. Pharmacol. Biochem. Behav. *24*, 299-304.

12. Gibson, C.J. and Wurtman, R.J. (1977) Physiological control of brain catechol synthesis by brain tyrosine concentration. Biochem. Pharmacol. *26*, 1137-1142.

13. Johnson, D.J. and Anderson, G.H. (1982) Prediction of plasma acid concentration from diet amino acid content. Am. J. Physiol. *243*, R99-R103.

14. Faust, I.M. (1981) Signals from adipose tissue. In: The Body Weight Regulatory System: Normal and Disturbed Mechanisms (Cioffi, L.A., James, W.P.T., Van Itallie, T.B. eds.) pp. 36-43, Raven Press, New York.

15. Fowler, C.J. and Ross, S.B. (1984) Selective inhibitors of monamine oxidase A and B: biochemical, pharmalogical and clinical properties. Medicinal Res. Rev. *4*, 323-358.

16. Iverson, L.L. and Glowinski, J. (1966) Catecholamine regional metabolism in the rat brain. J. Neurochem. *13*, 655-669.

17. Slotnick, B.M. and Leonard, C.M. (1975) A Stereotoxic Atlas of the Albino Mouse Forebrain. DHEW Publication No. (ADM) pp. 75-100, U.S. Department of Health, Education and Welfare, Rockville, Maryland.

18. Bioanalytical Systems Inc., (1980) Tissue catecholamines: simultaneous assay for norepinephrine, epinephrine and dopamine. LCEC Application Note 12.

19. Markwell, M.K., Haas, S.M., Bieber, L.L. and Tolbert, N.E. (1978) A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal. Biochem. *87*, 206-210.

20. Jones, B.N. and Gilligan, J.P., (1983) O-Phthaldialdehyde precolumn derivatization and reversed-phase high-performance liquid chromatography of polypeptide hydrolysates and physiological fluids. J. Chromatog. *266*, 471-482.

21. Association of Official Analytical Chemists (AOAC) (l980) Official methods of analysis. 13 ed. AOAC, Washington.

22. Gill, J.L. (1978) In: Design and Analysis of Experiments in Animal and Medical Sciences, Vol. 1., pp. 95-107, Iowa State University Press, Ames, Iowa.

23. Steel, R.G.D. and Torrie, J.H. (1983) Principles and Procedures of Statistics: A Biometrical Approach, 2nd ed., pp. 106-107, McGraw-Hill, New York.

24. Johnston, J.L., Romsos, D.R. and Bergen, W.G. (1986) Reduced brain norepinephrine metabolism in obese (*ob/ob*) mice is not normalized by tyrosine supplementation. J. Nutr. *116*, 435-445.

25. Nair, K.S., Halliday, D. and Garrow, J.S. (1983) Thermic response to isoenergetic protein carbohydrate or fat in lean and obese subjects. Clin. Sci. *65*, 307-312.

26. Jhanwar-Uniyal, M., Fleischer, F., Levin, B.E. and Leibowitz, S.F. (1982) Impact of food deprivation in hypothalamus α-adrenergic receptor activity and norepinephrine (NE) turnover in rat brain. Soc. Neurosci. Abstr. *8*, 711.

27. Leibowitz, S.F., Roossin, P. and Rosenn, M. (1984) Chronic norepinephrine injection into the paraventricular nucleus produces hyperphagia and increased body weight in the rat. Pharmacol. Behav. *21*, 801-808.

28. Shor-Posner, G., Azar, A.P., Insinga, S. and Leibowitz, S.F. (1985) Deficits in the control of food intake after hypothalamic paraventricular nucleus lesions. Phys. Behav. *35*, 883-890.

29. Jhanwar-Uniyal, M., Darwish, M., Levin, B.E. and Leibowitz, S.F. (1987) Alterations in catecholamine levels and turnover in discrete brain area after food deprivation. Pharmacol. Biochem. Behav. *26*, 271-275.

30. Meek, J.L. and Neff, N.H. (1973) The rate of formation of 3-methoxy-4-hydroxyphenylethyleneglycol sulfate in brain as an estimate of the rate of formation of norepinephrine. J. Pharmacol. Exp. Ther. *184*, 570-575.

31. Li, P.P., Warsh, J.J. and Godse, D.D. (1984) Formation and clearance of norepinephrine glycol metabolites in mouse brain. J. Neurochem. *43*, 1425-1433.

32. Lovenberg, W. and Victor, S.J. (1974) Regulation of tryptophan and tyrosine hydroxylase. Life Sci. *14*, 2337-2353.

33. Macho, L. Kvetnansky, R., Culman, J. and Fickova, M. (1986) Neurotransmitter levels in the hypothalamus during postnatal development of rats. Exp Clin. Endocrinol. *88*, 142-150.

34. Lorden, J.F., Oltmans, G.S. and Margules, D.L. (1976) Central catecholamine turnover in genetically obese mice (*ob/ob*). Brain Res. *117*, 357-361.

35. Papeschi, R. (1977) The functional pool of brain catecholamines: its size and turnover rate. Psychopharmacol. *55*, 1-7.

36. Glowinski, J. (1973) Some characteristics of the 'functional' and 'main storage' compartments in central catechominergic neurons. Brain Res. *62*, 489-493.

37. Oltmans, G.S. (1983) Norepinephrine and dopamine levels in hypothalamic nuclei of the genetically obese mice (*ob/ob*). Brain Res. *173*, 369-373.

38. Schweiger, U., Warnhoff, M. and Pirke, K.M. (1985) Norepinephrine turnover in the hypothalamus of adult male rats: alteration of circadian patterns by semistarvation. J. Neurochem. *45*, 706-709.

39. Brodie, B.B., Costa, E., Dlabec, A., Neff, N.N. and Snookler, H.H. (1966) Application of steady-state kinetics to the estimation of synthesis rate and turnover time of tissue catecholamines. J. Pharmocol. Exp. Ther. *154*, 493-498.

40. Levin, B.E., Triscari, J. and Sullivan, A. (1985) The effect of diet and chronic obesity on brain catecholamine turnover in the rat. Pharmacol. Biochem. Behav. *24*, 299-304.

41. Gibson, C.J. and Wurtman, R.J. (1977) Physiological control of brain catechol synthesis by brain tyrosine concentration. Biochem. Pharmacol. *26*, 1137-1142.

42. Pardridge, W.M. and Oldendorf, W.H. (1975) Kinetic analysis of blood-brain barrier transport of amino acids. Biochim. Biophys. Acta *401*, 128-136.

43. Fukagawa, N.K., Minakev, K.L., Young, V.R. and Rowe, J.W. (1986) Insulin dose-dependent reductions in plasma amino acids in man. Am. J. Physiol. *250*, E13-E17.

III. APPENDICES

Appendix 1.

Assay of Norepinephrine

1. Previously weighed frozen tissues were homogenized in 1 ml 0.4 M perchloric acid ($HClO_4$), 20 µl 0.2 M ethylenediamine tetraacetic acid (EDTA), 10µl 1.0 M sodium bisulfite ($NaHSO_4$), and 100 µl 0.0007 M 3,4-dihydroxybenzylamine (DHBA) as an internal standard.

2. Homogenates were centrifuged at 9000 rpm for 10 min and aliquots of the supernatant were transferred to vials to which 35 mg acid-washed alumina and 2 ml 2 M tris buffer (pH 8.6) were immediately added.

3. The samples were agitated for 20 min. with a mechanical shaker and the supernatants were removed by aspiration.

4. The alumina was washed three times with 3 ml HPLC grade water and the NE and DHBA were then eluted from the alumina with 200 µl 0.2 M $HClO_4$.

Appendix 2

Protein Determination of Hypothalamic Nuclei

Materials

- REAGENT A
  2.0% sodium carbonate (Na_2C0_3)
  0.4% sodium hydroxide (NaOH)
  0.16% sodium tartrate
  0.16% sodium dodecyl sulfate (SDS)
- REAGENT B
  4.0% copper sulfate pentahydrate ($CuS0_45H_20$)
- REAGENT C
  100 parts of reagent A mixed with 1 part reagent B
- REAGENT D
  Folin-Ciocalteu phenol reagent

1. Three ml of Reagent C were placed in culture tubes. Crystallized bovine serum albumin (BSA) was used as a standard.
2. Protein samples were placed in culture tubes and incubated at room temperature for 60 min.
3. Three hundred µl of Reagent D were added to each tube and mixed vigorously by vortexing.
4. Samples were incubated at room temperature for 45 min.
5. Absorbance was read at 660 nm ± .002 A on a mass spectrophotometer. (Unicam SP 1800 Ultraviolet Spectrometer, Beckman Instruments Inc., Fullerton, CA).

Appendix 3.

Analysis of Neutral Amino Acids by HPLC (o-Phthaldialdehyde derivatives)

Chromatography system

Separation and quantification of individual neutral amino acids was accomplished with high performance liquid chromotography with fluormetric detection. Samples were mixed using a modified Technicon autosampler and a Chemlab perstaltic pump with a stainless steel mixing tee. Samples were mixed 1:1 with the fluorescence reagent prior to injection and delay time was 12 seconds. Samples were injected using a Valco autoinjector valve equipped with a 20µl loop. The column used was a Supelcosil 3 µ LC-18 reverse phase column and a guard column packed with Supelco LC-18 reverse phase packing. Chromatographic peaks were recorded using a Fisher recorder, and integration was accomplished using a Hewlett Packard 3353 data system with a Hewlett Packard 18652A A/D convertor.

Preparation of Reagent for Fluorescence

One gram of o-phthaldialdehyde (OPA) was dissolved in 25 ml methanol; 224 ml 0.04 M sodium borate buffer (pH 9.5) were added along with 1.0 ml 2-mercaptoethanol and 20 ml Brij 35.

Gradient Conditions

Solvent A was prepared by adding 11.5 ml glacial acetic acid and 8.0 g of sodium hydroxide to 1600 ml deionized water. The pH was adjusted to 7.2 with 5 M sodium hydroxide. 180 ml methanol and 10 ml tetrahydrofuran were added and the volume was adjusted to 2 litres with deionized water. Solvent B was methanol. Flow rate was 1.1 ml/min.

Preparation of samples

Plasma samples

To 25 µl plasma, 200 µl ethanolamine (50 nmol/ml) and 25 µl water were added. Samples were vortexed and 500 µl 5% sulphosalicylic acid were added. Samples were centrifuged (3000 rpm, 15 minutes) and 500 µl saturated potassium borate and 500 µl water were added to the supernatant.